**S.A. SHALIN, A.A. KESKINOV**

# MONONUCLEAR ONCOGENESIS

MOSCOW

2010

Authors:
Sergey Alexeevich Shalin, surgeon, PhD, Associate Professor.
Anton Arturovich Keskinov, physician-researcher.

This book is devoted to topical issues of theoretical oncology: the mechanism of the “birth” of a malignant stem cell, the growth of the malignant focus and the development of the malignant process. Based on published data, our own observations and thinking, a universal and easily comprehensible theory has been developed where many of the accepted facts find their place. The proposed theory gives general ideas about the aetiology and pathogenesis of malignant tumours, thus creating the necessary basis for their further study and improvement.

The book is intended for students and teachers at medical and biological centres of learning, researchers as well as doctors of all specialties, who are interested in the problem of malignant diseases.

ISBN 978-5-902164-69-2

Dear Reader,
Given the importance of the problem under study and the novelty of the proposed theory, your own opinions on the material presented in the book are very welcome.

Comments and criticisms can be sent to:
Sergey A.Shalin
E-mail: schalin@mail.ru

**CONTENTS**

# INTRODUCTION

Oncogenesis is the multistage mechanism of the beginning, growth and development of the malignant process.

Given the above definition, oncogenesis consists of three key processes and every following process is the result of the previous one. Every one has its distinctions and oncogenesis can be terminated at any of them.

The most vexed question in contemporary oncology is the main or fundamental issue of the nature of the malignant process, as there are still discussions about the origins of malignant cells.

Here we do not hope to cover the problem of oncogenesis completely, and in principle this is impossible. Our objective is to

suggest a universal theory, which is simple to understand and where most of the recognised facts can be addressed.

# 1. THE MALIGNANT CELL

***Miller E., Miller J. (1966):*** *suggested a theory of nuclear-genetic mechanisms of multistage carcinogenesis, according to which the "birth" of a malignant cell takes place in two stages: initiation and promotion.*

Details of the nuclear-genetic mechanism of oncogenesis are available in related literature, so let us summarise and analyse its key provisions.

**First stage (initiation)** – following a carcinogenic impact (ionising radiation, endo- and exo-carcinogens and viruses) together with a relatively neutral injury of a genome, significant mutations in oncogenes and anti-oncogenes may occur. As the result of this, a range of distinctive abnormalities occurs at the levels of gene, chromosome and genome: amplifications (increase of gene abundance), deletions, insertions, translocations, micro-mutations (point substitutions, micro-deletions and micro-insertions) etc.

**Conditions for initiation:**

- the initiator's (ionising radiation, endo- and exo-carcinogens and viruses) application has to be single and short-term, and the occurrence of tumour formation depends on the dose of the initiator – the stronger the dose, the surer the outcome;

- initiation occurs only during the mitosis of a cell, i.e. in the zone of natural intensive proliferation of somatic cells;

- initiation is more likely to occur in the area of chronically amplified proliferation stimulated by external or internal impact, i.e. certain chronic processes in the host have to maintain constant enhanced proliferation of somatic cells;

- initiation is irreversible, i.e. abnormalities occurring at the levels of gene, chromosome and genome cannot be restored back to normal conditions;

- initiation has to stop completely before the promoter can take effect, i.e. a change in a cell's state is necessary: initiation has to happen in one condition, but the further impact (promotion) can only be effected when the cell with a changed genotype is already in different living conditions and microenvironment;

- given that a malignant cell has embryonic features, the proliferating zone has to have its beginning in the embryonic period of development of the organism and also in conditions similar to embryonic ones during the period of transformation of a normal somatic proliferating cell into a malignant cell;

- it is well known that malignant cells have a different level of potency: from unipotent to pluripotent, i.e. the potency level of the cell precursor during its transformation into a primary malignant stem cell has to be high enough – unipotent or pluripotent.

- ***Schimke R.T. (1981****): the minimal occurrence of malignant neoplasm, which can be inherited, is 7% on average (1-15%, according to different authors, for different oncological diseases), i.e. on average 7% of cases lead to the emergence of malignant diseases, which are inherited by "blood" relatives*. Here the stage of initiation is not necessary. Irreversible changes of genotype in nuclear DNA have already been inherited.

**Thus** a single and short-term carcinogenic impact leads to irreversible genotype changes of a proliferating somatic cell's nuclear DNA. However, initiation itself is not enough for the "birth" of a malignant stem cell.

**Second stage (promotion)** – a genotype-changed cell is affected by the promoter in conditions which differ from the initial state. In the first instance the cell's membrane and cytoplasm are affected. Structural alterations of the cell membrane and chemical changes in the cell's cytoplasm affect the manifestation of genotype abnormalities of nuclear DNA – the epigenetic mechanism.

**Conditions for promotion:**

- promotion is effective only after initiation and, moreover, after the effect of the initiator has completely terminated, i.e. the

initiated cell has to be in different living conditions and microenvironment;

- the interval between initiation and promotion does not affect the final occurrence of malignant neoplasm, i.e. the life time of an initiated cell can be variable, but it is necessary for it to be as long as possible (months, years);

- the promoter has to impact on the initiated cell incessantly and for a long time, i.e. the initiated cell with a prolonged life-cycle has to be in certain isolated conditions in which an aggressive impact on it can continue for a relatively long period (months, years);

- the promoter can affect the initiated cell in different ways, concerning: the structure of the cell's membrane with alteration of its selective permeability; the chemical state of its cytoplasm; its differentiation; its ability to block the intercellular linkage etc.;

- promotion is reversible in the beginning stage, i.e. early manifestations of the promoter's effects can disappear and the cell will return to its initiated state.

- ***Warburg O.H. (1956****): malignant cells emerge by selection during abnormalities of normal cells' respiration in low oxygen conditions or in an oxygen-free medium. The majority of cells die in such conditions, but those which during the selection have changed their metabolism for intensive glycolysis, i.e. oxygen-free energy release – survive, reproduce and make a malignant focus in a number of generations.*

**Thus,** the proliferating somatic cell, which has genotype alteration of nuclear DNA, gets into the "super circumstances" where it is aggressively and incessantly affected in oxygen-free conditions for a long time (months, years). As a result it experiences epigenetic changes – structural alterations of the cell's membrane and chemical changes in the cytoplasm.

At the present time it is considered that genotype and epigenetic alterations of a proliferating somatic cell prepare the mechanism of transformation into a malignant stem cell, and at the same time launch it. The precondition (sine qua non) for a

transformation mechanism to work is sufficient insulation from the influence of the host.

# 2. MALIGNANT DISEASES

All human malignant diseases are divided into two main groups: haemoblastosis and solid tumours. The principle of division is based on different localisation of malignant process and some similarity between malignant cells and normal cells of the microenvironment.

Despite substantial information and data being now available, the question of the origins of malignant neoplasm is yet disputable. Earlier on there were talks about histogenesis – tissue origins, now we can speak about cytogenesis – the cell origins of malignant neoplasm.

It is considered that the most studied is cytogenesis of malignant diseases of haematopoietic and lymphoid tissue, which is based on the theory of stem and semi-stem haematopoietic precursors. Cytogenesis of solid tumours is not yet well studied and there is no clear understanding of which precursors emerge from the malignant cells. That is why we will mainly cover issues of solid tumours, where possible drawing an analogy with haemoblastosis.

**Common symptoms of haemoblastosis and solid tumours**

1. Ethiology: chemical agents (endo- and exo-carcinogens), ionising radiation and viruses.

2. Pathogenesis: genotype alteration of nuclear DNA and epigenetic alterations – damage of a cell's membrane structure and chemical processes in cytoplasm of the cell following the emergence of new features or copying the information from the virus's DNA/RNA over to the cell's genome, which eventually leads to the appearance of malignant growth symptoms.

3. Diagnostics: clinical, laboratory and instrumental methods of examination with compulsory morphological verification of the diagnosis (histological and cytological studies).

4. Complications: inflectional, thrombotic, abnormality of the normal mechanism of osteogenesis, gastrointestinal complications (nausea, vomiting, hiccough, constipation, diarrhoea and mucositis), intoxication and psychological changes (anxiety, depression, aggressiveness and suicide).

5. Principles of pathogenic therapy: interventions, which depress the proliferation of malignant cells (X-raying, chemotherapy, hormonotherapy and immunotherapy), vitamin therapy, auxiliary therapy (blood transfusion, relief of infection, treatment of thrombosis and bleeding) and bone marrow transplantation.

6. Causes of death: cachexia, secondary infection, severe anaemia, thromboembolismic complications, voluminous bleeding and haemorrhage.

7. Basic symptoms of malignancy: all features are inherited, the principle of malignant succession is in force, uncontrolled cell division, invasive growth and metastasis.

**Thus** the general similarity of haemoblastosis and solid tumours are set at the genetic level.

## Differences between haemoblastosis and solid tumours

Let us consider each group of malignant diseases separately and also split haemoblastosis into two sub-groups: leucosis and lymphoma.

Based on studies of the onsets of all malignant diseases, we can draw the following conclusions:

**1. Leucosis** is a numerous and heterogeneous group of malignant diseases, which emerge from haemopoietic (blood forming) cells and affect red marrow.

- the precursors of malignant stem cells are pluripotent or unipotent stem cells of the foci of myelo- or lymphopoiesis in the red marrow;

- both stages (initiation and promotion) of the “birth” of a malignant stem cell take place in the same location – in the red bone marrow;

- the basis of a malignant stem cell “birth” is the block of differentiation and transformation of a pluripotent or unipotent stem cell of myelo- or lymphopoiesis;

- the mechanism of a malignant stem cell “birth” lies in the genotype and epigenetic changes of a pluripotent or unipotent stem cell of myelo- or lymphopoiesis following the carcinogenic impact;

- the malignant process starts from the “birth” of one malignant stem cell, which then forms a clone of malignant cells;

- the disease is manifested through growth of the malignant process in the red marrow, but at that time a primary malignant focus is absent;

- the malignant process develops by the proliferation of malignant cells within the red marrow, and by haematogenous and lymphogenous spread in the host body;

- the malignant process impacts haemopoiesis, homeostasis, immunity etc.

**2. Lymphoma** is a group of malignant haematological diseases of lymphatic tissue characterised by malignant transformation of lymphoid cells;

- the precursors of malignant stem cells are pluripotent or unipotent stem cells of lymphopoiesis located in the red marrow;

- the first stage (initiation) of the “birth” of a malignant stem cell takes place in the red marrow, the second stage (promotion) in the location of the primary malignant focus;

- the basis of a malignant stem cell “birth” is a block of differentiation and transformation of a pluripotent or unipotent stem cell of lymphopoiesis;

- the mechanism of a malignant stem cell “birth” is the genotype and epigenetic changes of a pluripotent or unipotent stem cell of lymphopoiesis following the carcinogenic impact;

- the malignant process starts from the “birth” of one malignant stem cell, which then forms a clone of malignant cells;

- the malignant process is manifested through forming the primary malignant focus located in the lymph nodes (nodal involvement) or in any other organs and tissues (extra nodal involvement);

- the malignant process is developed by lymphogenous spread in the host body, sometimes cells of lymphoma are detected in the

blood, but usually they tend to form thick tumours in the lymphatic system or in the internal organs (liver, stomach, nervous system or in other places);

- the malignant process impacts haemopoiesis, homeostasis, immunity etc.

**3. Solid Tumours** are the largest in quantity, heterogeneous group of malignant diseases, which develop through multi-stage malignant transformation of a normal proliferating somatic cell into a malignant stem cell:

- the origins of malignant cells are not known, as the precursor of the malignant stem cells is not known. It is supposed that the precursors are normal somatic proliferating cells - cambial cells, which are located in the area of the crypts' floor, glands' neck, periosteum and perichondrium, along the blood vessels' flow and also in the area of intense restorative proliferation;

- it is not known where and how both stages (initiation and promotion) of the "birth" of a malignant stem cell take place. It is supposed, in the area of intensive proliferation of cells;

- also it is not known what process underlies the "birth" of a malignant stem cell. It is supposed that a normal somatic proliferating cell transforms into the malignant stem cell;

- the mechanism of the "birth" of a malignant stem cell is not known. It is supposed that following the carcinogenic impact the genotype and epigenetic changes of a normal proliferating somatic cell take place, and these are a launching mechanism for its transformation into the malignant stem cell;

- a malignant focus starts with the "birth" of one malignant stem cell (in 80% cases), two or more malignant stem cells (20%), which then form a clone of malignant cells;

- the malignant process is manifested through forming the primary malignant focus, which can be located in different organs and tissues, and it increases by proliferation of cells, appositional and invasive growth;

- the malignant process is developed by the haematogenous and lymphogenous spread in the host body while forming the secondary foci – metastases;

- the malignant process impacts haemopoiesis, homeostasis, immunity etc.

**Thus,** the contemporary notions:

1. The difference between haemoblastosis (leucosis and lymphoma) on the one hand and solid tumours on the other, is in the mechanism of the “birth” of a malignant stem cell;

- in the case of haemoblastosis, following the carcinogenic impact the genotype and epigenetic changes of a pluripotent or unipotent stem cell of myelo- or lymphopoiesis, a block of differentiation and its transformation into a malignant stem cell take place;

- it is supposed that in the case of solid tumours, following the carcinogenic impact genotype and epigenetic changes of a normal proliferating somatic cell take place, which are a launching mechanism for its transformation into a malignant stem cell.

2. The difference between leucosis on the one hand and lymphoma and solid tumours on the other, is in the manifestation of the malignant process:

- in the case of leucosis, the disease manifests itself by the affection of the red marrow, but the primary malignant focus is not formed. The “birth” of a malignant stem cell requires 2-4 genotype alterations of the nuclear DNA of a pluripotent or unipotent stem cell of myelo- or lymphopoiesis. Epigenetic alterations are of secondary importance, that is why changes in the living conditions and microenvironment of the precursor are not a prerequisite;

- in the case of lymphoma and solid tumours, the disease manifests itself by necessary forming of the primary malignant focus. The “birth” of a malignant stem cell requires 7-8 genotype alterations of the nuclear DNA of the precursor of the malignant stem cell. The genotype and epigenetic alterations are of equal importance, that is why changes in the living conditions and microenvironment of the precursor are a prerequisite. The large number of genotype alterations of nuclear DNA and the equally important genotype and epigenetic alterations determine the length of pre-clinical evolution of the disease.

3. The features of tumorous growth of lymphoma are common with those of solid tumours – they form the primary malignant focus and metastasis, as well as with leucosis; they can form the states, which are analogous to lymphoid leucosis. That is why the lymphoma is considered as an intermediate variant of the malignant process development.

4. Given that in the case of haemoblastosis the first stage (initiation) of the “birth” of a malignant stem cell takes place in

the red marrow, it would be logical to suppose that in the case of solid tumours the first stage (initiation) of the precursor of a malignant stem cell also takes place in the red marrow. So the following second stage (promotion) occurs in the organs and tissues where the “birth” of a malignant stem cell takes place and it forms the primary malignant focus.

# 3. PREREQUISITES FOR MONONUCLEAR ONCOGENESIS

To create a theory of “mononuclear oncogenesis” as a natural mechanism for the origins, growth and development of the malignant process, we think it is possible to query the existing theory of the origins of the primary malignant stem cell of solid tumours from cambial cells of tectorial or glandular epithelium. And the following question - from which cell and/or tissue the substrate malignant stem cells of solid tumour originate, is still debatable and has not yet determined exactly the range of cells which can claim the role of the precursor of the primary malignant stem cell.

**So here we have two questions:**

**Question 1:** On the basis of what facts can we query the origins of a primary malignant stem cell from the cambial cells of tectorial or glandular epithelium?

**Answer:**

1. Theoretically any somatic cell can transform into a malignant cell. However, the processes of transformation in vitro should not be equalled with the cell oncogeneicity in vivo, as the transformation of a normal cell into a malignant cell is a process initiated at the molecular level and it includes sequential work of several groups of genes, with oncogenes playing the key role of “launching” these processes.

2. The malignant process emerges not immediately, it is preceded by pathological alterations, which under certain circumstances lead to the onset of a malignant disease. It is related to the processes which, characterised by the tissue

growth and such conditions, are called pre-cancerous. The conception of a “pre-cancer” covers alterations of clinical, morphological (structural), cytological (cellular) and biochemical character. Often these are chronic inflammatory and dystrophic alterations.

3. At the onset and development of the malignant process, not only the local pathological alterations of the tissue, but also systemic disease of the organism should be taken into account. A tumour emerges in one or another organ or tissue, in this or that place, starting with the onset of a tumour “germ” or malignant “embryo”, which in the beginning consists of a small group of cells, which have altered and are altering further. Emergence of such an “embryo” can be preconditioned by various impacts, which have an effect not only locally but on the entire organism as well.

4. ***Sharai L.A. and co-authors (1961):*** *experiment on a cat’s stomach – a chronic inflammatory and regenerative process similar to the state of the epithelium, which can be observed in the case of human stomach carcinoma (cats never have stomach carcinoma), was reproduced by daily introduction of milk or meat broth at the temperature of 59-61C into the cat’s stomach during the course of a year. However, after the aggressive impact had been terminated, the stomach epithelium gradually normalized. Conclusions: hyperplasia and anaplasia of the epithelium do not lead to its transformation into a malignant cell.*

5. ***Cornil A.V. (1908):*** *described the similarity of invasive carcinoma cells with the cells of ducts’ epithelium.* However, the assumption that malignant cells emerge from the epithelium cells has been discussed right up to our time. Today it can be said unambiguously that hyperplasia and metaplasia of the epithelium do not lead to malignant transformation. And epithelial dysplasia is already considered as a malignant epithelium, whose origins are not clear.

6. Knowledge about the earliest alterations in epithelium cells as the precursors of malignant cells is limited by the consideration of the absence of a pre-tumour state, which would be common for all sorts of cancer, and by the scant data about the earliest stages of various malignant diseases.

7. Atypical surface epithelium is an unstable short-term structure; it is easily rejected and then often does not form again, i.e. the evidence of regenerative ability is very poor. In consequence, malignantly transformed parts of the mucous membrane are often deprived of the epithelium lining and a lot of detritus, produced by dead elements, accumulates on the wound surface.

8. ***Turkington (1972):*** *in normal conditions, under the influence of hydrocortisone and prolactin, the epithelium cells of the mammary gland differentiate into secreting alveolar cells. In the case of mouse breast carcinoma, the malignant cells do not respond to these hormones of cell differentiation.*

9. ***Reichlin N.T. (1975):*** *the only source for the emergence of malignant cells is the cambial cells (stem cells or cell-precursors), retaining to some extent the ability to differentiae in the process of malignant transformation*. However, the epithelium cells do not have any one feature which has malignant cells and no one function of the epithelium (tectorial, protective and exocrine) has passed to the malignant cells.

10. In the case of the malignant transformation of an adult organism's normal tissues, the gradual transition from the normal tissues to malignantly transformed ones has not been observed; and histological and cytological methods have failed to find the transition of the cells from normal cells of the microenvironment to malignant cells.

11. Stomach carcinoma: at the present time it has not been established what should be considered as the beginning of the malignantly transformed growth in the mucous membrane, and while this process is going on, what is the structure of the epithelium. The main symptoms of epithelium differentiation - the secreting activity and heteropolarity - are disordered while the malignant process is going on. Malignant cells of stomach carcinoma very frequently express factors which inhibit migration of macrophages, and this is not usual for normal epithelium cells of the stomach's mucous membrane. Primary malignant cells are located within the mucous membrane; the secondary malignant cells grow into the mucous membrane from the sub-mucous one.

12. Lung carcinoma: from experiments it is considered there are two sources of malignant cells of primary carcinoma of the lung – the epithelium of bronchi and alveolar lining, though it is not possible to differentiate malignant cells of both neoplasmata.

13. In “pre-cancerous” liver the focal niduses of regeneration emerge, which consist of morphologically and histochemically homogeneous hepatocytes. The nodules closely resemble the clonal haematopoietic colony-formation in the spleen of radiation-exposed animals. There is no evidence of their emergence from the oval cells or young hepatocytes. After the termination of the carcinogenic impact the hyperplastic nodules lose their insulation and enter the structure of the normal liver. What precursors forms the basis for the clonal haematopoietic colony-formation in the “pre-cancerous” liver is not known.

14. Primary liver carcinoma: it is considered that malignant cells can issue from the epithelium cells of parenchyma - hepatoma (hepatocellular carcinoma) or from the epithelium of the bile ducts - cholangioma (cholangiocellular carcinoma), though it is not possible to differentiate malignant cells of both neoplasmata.

15. The genesis of malignant cells in the skin, oesophagus and rectum goes on quite similarly and the malignantly transformed states of these cells are not much diversified.

16. In metastases of melanoma the cell elements show a high level of differentiation abnormality; a pigmentary inclusion, melanin, remains the decisive symptom.

17. The rates of growth of the malignant focus are notable for an extremely wide range and they do not depend on their affiliation to organs, their morphological structure and the number of mitoses in the malignant tissue.

18. The “germination” of malignant cells is advanced by the media, which significantly differ from the current environment of the normal cells’ habitation. Undoubtedly, the first malignant cells do not have such a level of perfection, which is a feature of the normal cells around them. However, malignant cells are perfect in their own way, and further on in the result of malignant progression the level of their perfection evolutionally increases.

19. It is considered that malignant cells of solid tumours in different organs and tissues originate from different germinal layers; however, the principles of the genesis, growth and development of the malignant process are completely identical.

20. Based on the morphological data, it is usually pointed out that the malignant neoplasm, which is particularly different from tissues of the microenvironment, is also the most malignant one.

21. The malignant process takes place according to a certain programme and the common rule is its individual structure's known constancy during all periods of emergence, growth and development.

22. Specific morphological features of such forms of malignant neoplasm are mainly determined in the malignant "embryo". There are numerous variations of this rule with the evolution of the structure of malignant neoplasm, either to a more differentiated or (more often) to a less differentiated structure.

23. There are phenotypic and genotype differences between malignant cells of leucoses, lymphoma and solid tumours, between malignant cells of the same group and even between malignant cells in one malignant neoplasm, but according to key characteristics all malignant cells are the same.

24. Important and uncontested assertions:
- malignant cells have more similarity between themselves than normal cells have between themselves;
- malignant cells have fewer differences between themselves than the differences between malignant cells and normal cells;
- normal cells have fewer differences between themselves than the differences between normal cells and malignant cells;
- the basic principles of the "germination" of malignant cells, the growth of the primary focus and the development of the malignant process in different organs and tissues are completely identical.

25. And many other issues.

**Thus,** given the above, some doubts can be expressed regarding the origins of the primary malignant stem cell from the cambial cells of tectorial or glandular epithelium.

**Question 2:** What cell can bid for the role of the “common beginning” or the cell-precursor of the primary malignant stem cell of solid tumours?

**Answer:**

The origins of the “germination” of the primary malignant stem cell of solid tumours should not be sought among local cells of the primary malignant focus’s surroundings, as it has been done unsuccessfully for decades, but in the long evolutionary pre-history and in the embryonic period of the host-body development, with consideration of the environment, which is significantly different from the previous environment of the precursor’s habitation.

Analysing all cells of the human body, cells which have the following key features should first be selected:

1. They are somatic proliferating cells, which have a lengthy life cycle (months, years).

2. They are autonomous, can easily travel all over the host-body, penetrate and migrate in organs and tissues.

3. They have an ability to influence various vital processes: haemopoiesis, homeostasis, immunity, proliferation, maturation and differentiation of cells etc.

The only cells in the human body which have the above features are the cells of the blood system. Of all cells of the blood system only mononuclear cells can bid for the role of the “common beginning” or the cell-precursor of the primary malignant stem cell of solid tumours.

**The above assertion is supported by the following:**

- only mononuclear cells (promonocytes and monocytes) are somatic and proliferating cells;

- only mononuclear cells have a lengthy life cycle (months and years), all other cells of the blood are short-lived – they quickly finish their life cycle;

- only mononuclear cells are sufficiently autonomous: they can easily travel all over the host-body in the blood flow, penetrate and migrate into various organs and tissues;

- only mononuclear cells (promonocytes and monocytes) in the red marrow and in the bloodstream are the intermediate variant

of development and in the tissues they transform into macrophages; all the other cells of the blood are dead-locked variants having no chance for development;

- only mononuclear cells are powerful enough to influence various vital processes which are going on in the macro-organism: haemopoiesis, homeostasis, immunity, proliferation, maturation and differentiation of cells etc;

- only mononuclear cells can take the phenotype of the cells in the microenvironment – the mesenchymal-epithelial transition.

**Thus,** the mononuclear cell is a cell which can bid for the role of the “common beginning” or the cell-precursor of the primary malignant stem cell of solid tumours.

# RÉSUMÉ

Discussing this issue is a very difficult task as many known facts are not univocal and they can be interpreted in different ways.

# CHAPTER I

# FORMATION OF THE “PRE-TUMOUR” BED

The emergence of the malignant process is preceded by the “pre-tumour” diseases of the organism in general and pathological alterations of local tissues in particular. This process is accompanied by the structural reconstruction of the organs and/or the tissues and by the formation of optimal conditions for the emergence, growth and development of the malignant process and unfavourable conditions for normal cells and tissues of the microenvironment.

## 1. PRE-TUMOUR DISEASES OF THE ORGANISM

Pre-tumour diseases of the organism is a general name for congenital and/or acquired alterations of tissues, which advance to the emergence, growth and development of the malignant process.

## Distinctions

From the clinical point of view there are obligatory and optional pre-tumour diseases:

1. Obligatory pre-tumour diseases – pathological alterations of tissues conditioned by genetic or congenital factors, which definitely transform into malignant disease: familial polyposis of the large intestine, xeroderma pigmentosum, Bowen's dermatosis, adenomatous polyp of the stomach, some kinds of mastopathy and some benign tumours. The time of emergence of malignant neoplasm depends on hereditary factors and the influence of external factors.

2. Optional pre-tumour diseases - pathological alterations of tissues not linked with hereditary or congenital abnormalities, in the background of which a malignant disease can emerge: chronic inflammatory diseases and foci of proliferation generated by hormonal reconstruction, after-burn scars, non-healing ulcers, cervical erosions, polyps and senile keratosis. And the longer the optional pre-tumour diseases exist, the more chance of the emergence of malignant neoplasm.

## Influences

The state of the enzymatic, hormonal and immune systems of the organism influence the emergence and development of malignant disease:

1. Allergy – the allergisation of the organism affects negatively its resistance to the emergence of the malignant process. However, if the malignant neoplasm has emerged, the state of the organism and the prognosis is better in those cases where the organism is able to make allergic reactions, rather than in those cases where it is not able to do this. Such disparity is explained by the organism's retaining, at least to some extent, the ability to make immune reactions, which provides anti-tumour protection.

2. Immunity – the decrease of the macro-organism's resistance to the emergence of malignant disease during the immunity's response to other diseases combines with the decrease of the cell and humoral autoimmunity, and also with interlocking immune mechanisms of anti-tumour protection. In old age, in ontogenesis the immune response proceeds incompletely, that is why malignant diseases emerge most often in this period.

3. Inflammation – irritation, wound and functional disorder, which takes place during inflammation as well as auto-immune processes, which are proceeding simultaneously, together with other factors also favour the emergence and development of the malignant process.

4. Metabolic syndrome – a range of metabolic, hormonal and clinical abnormalities, which are the factors of potential early emergence of arteriosclerosis and its cardiovascular complications. Primary insulin resistance and collateral systematic hyperinsulinemia underlie the pathogenesis of the metabolic syndrome. The metabolic syndrome is a combination of three or more chronic diseases: obesity, arterial hypertension, diabetes, dyslipidaemia, high cholesterol levels etc. The metabolic syndrome helps the development of inflammatory processes in the organism and increases the chances of the emergence of malignant neoplasm.

5. Reactivity and connective tissue - the cell's connective tissue elements (the reticuloendothelium system and the system of macrophages) take part in the formation of the organism's reactivity. They have the ability of phagocytic activity, provide intensive wound healing, and have barrier and antitoxic functions. Depression of higher nervous activity is accompanied by depression of the absorption function of the connective tissue elements, depression of the wound healing process, inflammation healing etc. The agitation of higher nervous activity stimulates the above mentioned functions of connective tissue cells.

**Risk factors**

Analysis of seven of the most widespread malignant diseases of the lungs, mammary glands, stomach, large intestine, prostate gland, cervix of the uterus and corpus uteri shows that there are the following risk factors in the basis of pre-tumour diseases:

- genetic or hereditary – possible in 1 - 15% of cases, if a susceptibility to the emergence and development of a malignant disease is transferred to the “blood” relatives;

- exogenous – dietary habits, infection, carcinogenic impact (ionising radiation, endo- and exo-carcinogens and viruses), which lead to rough morphological alterations of tissues followed by chronic inflammation, possible emergence and development of malignant disease;

- endogenous – ageing, the presence and development of chronic endocrine, hyperplastic and other diseases, which themselves can provoke chronic inflammation of tissues or advance to the transmission of an acute inflammation into a chronic one followed by the emergence and development of malignant disease.

**Thus,** a wide range of diseases is considered as pre-tumour: non-specific alterations of tissues due to inflammation, dystrophy or disharmony; benign tumour; developmental defects; age-related changes etc.

# 2. PRE-TUMOUR ALTERATIONS OF LOCAL TISSUES

Pre-tumour alterations of local tissues are non-specific pathological changes resulting from adaptation processes. However, this is not a stage of the consecutive transformation of a normal proliferating somatic cell into a malignant one, it is just a necessary preparation of tissues for making conditions in which a genotype-changed cell can acquire epigenetic alterations and transform into a malignant cell.

## 2.1 CHARACTERISTICS OF TISSUES

**Tissue** – a system of cells and their non-cellular derivatives, specialised in exercising certain particular functions. Tissues have specialised and poorly differentiated (cambial) cells, and also non-cellular structures: symplasts, intercellular substances, cuticular lumps etc.

**Epithelial tissue** - this is an aggregate of differones of polarly differentiated cells, which are tightly located like a layer on the

basic membrane at the border with the external or internal environment, and they also constitute the major part of glands in the organism (Fig. 1).

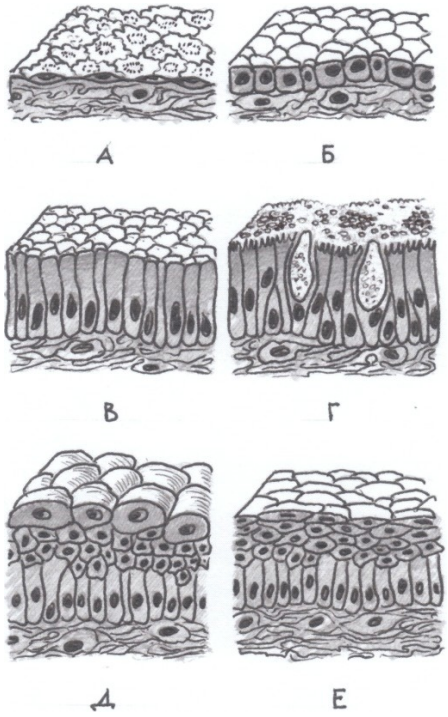



Fig. 1. The three-dimensional system of various types of the epithelium (A. Ham, J. Cormack, 1983; vol. 2, p. 9): A – squamous simple, B – cubical simple, C – cylindrical simple, D – cylindrical multirowed, E - transitional, F - stratified squamous non-keratinous.

Two groups of epithelial tissues can be distinguished:

1. The superficial epithelium – the border tissues, which are located on the surface of the body, mucous membranes of the viscera and on the secondary cavities of the body. They separate the organism and its organs from the environment, participate in the metabolism and protect relevant tissues of the organism against a range of external impacts.

2. The glandular epithelium – exercises the secreting function: it synthesizes and releases specific products (secretions), which are used in the processes going on inside the organism.

**The basement membrane** – formed as a result of activities of the epithelial cells, as well as cells of the underlying connective tissue (Fig. 2). Its thickness may be different; it is very thin and indistinct in the intestinal epithelium. The functions of the basement membrane are as follows:
1. Mechanical - anchorage of epithelial cells.
2. Trophic and barrier - selective transport of substances.
3. Morphogenetic – to ensure regeneration and to limit the possibility of invasive growth of the epithelium.

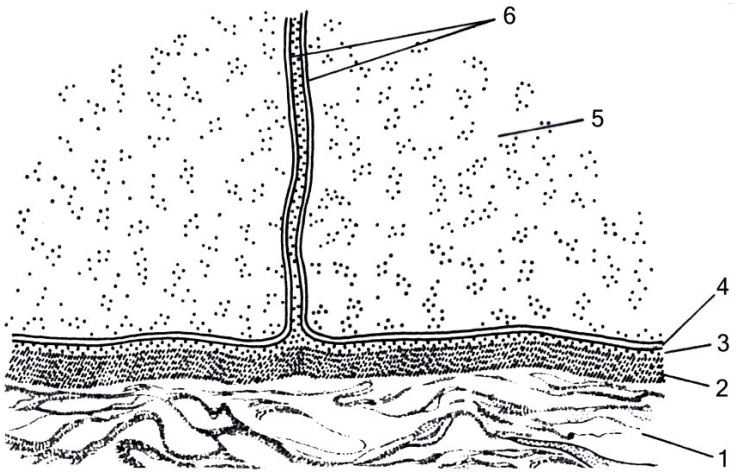



Fig. 2. The components of the basement membrane (A. Ham, J. Cormack, 1983; vol. 2, p. 54): 1 – argentophilic fibre, 2 - basal lamina, 3 - glycocalyx, 4 - plasma membrane of the basement cell, 5 - cytoplasm, 6 - lateral membranes of adjacent cells.

**The connective tissue** - is a major component of the stroma of any organ or tissue (Fig. 3). When in health or in disease it operates on the basis of mutually beneficial cooperation of the cellular elements among themselves, with the intercellular matrix and with the feedback from the parenchyma and blood cells; it can be seen as a **self-regulating system**.

Connective tissues - a semi-fluid mucopolysaccharide-protein environment in which there are the following subjects:

1. Various cellular elements: leukocytes, monocytes, macrophages, fibroblasts, mast cells, lymphocytes, plasma cells, etc.

2. Fibrous masses: collagen, elastic and argentophilic fibres.

3. Amorphous substance – a gelatinous substance, in which the fibres and connective tissue cells are submerged. It is a metabolic, integrative buffer and multi-component medium. It is composed of water, blood plasma proteins, inorganic ions, metabolic products of parenchymatous cells, soluble precursors of collagen and elastin, proteoglycans, glycoproteins and complexes formed by them.
Connective tissues are involved in maintaining homeostasis of the internal environment and differ from other tissues by **less need for aerobic** oxidation processes. The functions of connective tissue: trophic, protective, supporting (or biomechanical), plastic and morphogenetic.

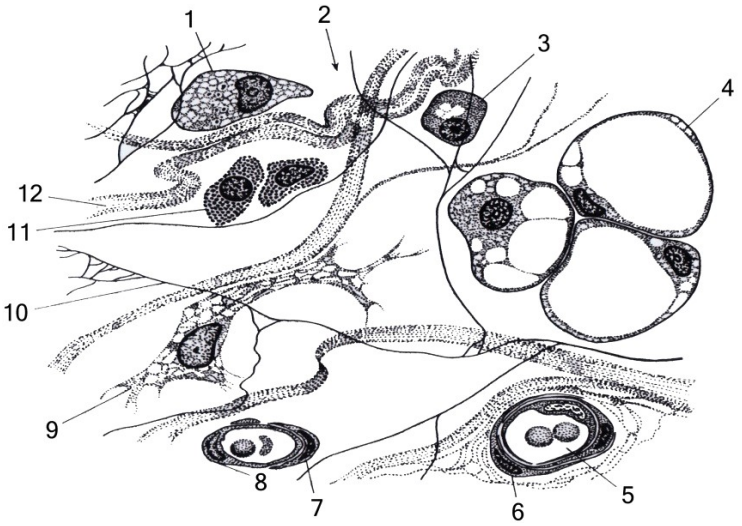



Fig. 3. Schematic image of the loose connective tissue (A. Ham, J. Cormack, 1983; vol. 2, p. 57): 1 - macrophage, 2 - amorphous intercellular substance, 3 - plasma cell, 4 - adipose cell, 5 - blood vessel, 6 - smooth muscle cell, 7 – pericyte, 8 - endothelial cell of the capillary, 9 - fibroblast, 10 - elastic fibre, 11 - mast cell, 12 - collagen fibre.

## 2.2 INFLAMMATION

***Kousmine C. (1956):*** *the cancer process is a specific form of inflammation.*

**Inflammation** is a protective-adaptive response of living systems, which emerged during evolution and which is manifested by a mass of local pathological processes of morphological, functional and physico-chemical nature in response to the impact of various stimuli.

It is known that the malignant process never occurs in healthy tissues, it is preceded by one or other chronic pathological alteration of local tissues, which are called "pre-tumour". In the first rank among chronic pathological alterations of local tissues which are of fundamental scientific and practical importance, is inflammation.

**Acute inflammation**

Acute inflammation is an immediate and early response to the damaging agent. It has three main components: alterations, disorders of microcirculation and proliferation.

**1. Alterations** – are tissue injuries: degenerative, destructive and necrobiotic phenomena. The destruction of tissue is caused by lysosome enzymes of the cells damaged during inflammation: leukocytes, monocytes, macrophages and parenchymatous cells of local tissues.

The consequence of activation of proteolysis, glycolysis and lipolysis is formation and release of large quantities of organic acids of the Krebs cycle, fatty acids, lactic acid, polypeptides and amino acids. The result of these processes is increase in osmotic pressure - hyperosmia. Increasing concentration of hydrogen ions (H+) leads to hyperoxia and acidosis. Destruction of cells is accompanied by accumulation of ions of potassium, sodium and chlorine, anions of phosphoric acid etc. in the inflamed tissue.

**2. Disorders of microcirculation -** in the early stages of inflammation a short spasm of arterioles occurs, followed by expansion of capillaries, arterioles and venules. Then, with the growth of the inflammatory process, the congestion and stasis of blood and lymph circulation appear. At the same time the liquid part of the blood (exudation) and forming elements - leukocytes (neutrophils), monocytes and lymphocytes, go out of the vascular bed into the inflamed tissue. Neutrophils located in the focus of inflammation help the body to clear the area of damage from infection and decay products of autologous cells, while the

mononuclear cells (monocytes, macrophages and lymphocytes) control the programme of reparative regeneration.

**3. Proliferation** – is the reaction of reproduction of the connective tissue's elements. Fibroblasts mitotically divide, creating colonies, and together with leukocytes form the inflammatory infiltration. Furthermore, in human peripheral blood there are pluripotent poorly differentiated cells of the connective tissue series, referred to as "peripheral blood fibrocytes" or as fibroblast-like cells, which are capable of migrating into areas of tissue damage and participating in the formation of both normal and pathological scars.

**Chronic inflammation**

Chronic inflammation is conceived as a long process (months or years), in which tissue damage, reactive alterations and scarring develop simultaneously.
Chronic inflammation may follow the acute inflammatory response, which does not destroy the injurious agent or proceeds without a clinically apparent acute stage.

The causes of chronic inflammation are diverse:
- different forms of phagocytic insufficiency;
- long-term stress and other states accompanied by a high concentration of catecholamines and glucocorticoids in the blood;
- repeated damage of a tissue or an organ;
- long-lasting infection and/or intoxication;
- pathogenic effect of immune self-aggression factors.

Pathogenesis of a chronic inflammation differs from an acute inflammation - it lasts long enough to have an immune response and regeneration. Most agents leading to chronic inflammation cause progressive and often extensive tissue necroses, which are accompanied by the replacement of fibrous tissue. Intensity of fibrosis in the tissues depends on the duration of chronic inflammations.

Chronic inflammation may be primary or secondary:
- if the inflammation is initially sluggish and has a long-term trend, it is called "primary-chronic";

- if after the acute period the inflammation becomes prolonged, it is referred to as “secondary-chronic”.

Chronic inflammation is characterised by a number of signs: granuloma, capsule, necrosis, and the predominance of monocytic and lymphocytic infiltration. Given the large number of mononuclear phagocytes and lymphocytes, chronic inflammation is designated as mononuclear-infiltrative, which reflects long-lasting reaction to injury, the destruction of tissues, and an attempt to restore damaged tissues through their replacement by the connective tissue or fibrosis and new growth of the small blood vessels (angiogenesis).

Chronic inflammation is diagnosed by its morphological features. It is distinguished from acute inflammation by the absence of the following basic signs: redness, swelling, pain and temperature rise. Active hyperaemia, fluid exudation and emigration of neutrophils are not expressed in the case of chronic inflammation.

**The outcome of inflammation**

There are several possible outcomes of inflammation:
- if not complicated by an acute inflammation the tissue returns to normal life through liquefaction and removal of exudate and cellular detritus by macrophages and the lymphatic system;
- if in the case of an acute inflammation the necrosis of tissue is full-blown, so its restoration occurs by regeneration or replacement of the connective tissue with the formation of a scar;
- if the damaging agent is not neutralised during the acute inflammatory response, an immune response develops, which then progresses to chronic inflammation;
- if the removal or neutralisation of the damaging agent is effected by chronic inflammation, the tissue also regenerates, usually by fibrosis.

In the process of proliferation the resorption of small blood clots and dead tissues (enzymatic cleavage, phagocytosis) occurs. Large defects of tissue, which result from the fibrinous-necrotic inflammation, are replaced by scar tissue. Small defects that appear between cells inside the local tissues (stroma) are lumens in the beginning and then they turn into **micro cavities**. Subsequently formed micro cavities become the “graveyard” for

the dead cells, because active processes of alteration and proliferation are not fast enough to quickly destroy and eliminate weak and damaged cells of local tissues and blood cells.

**Alterations in intercellular substance**

Physico-chemical changes in intercellular substance and tissue during inflammation are significant and manifested in the following abnormalities:
- increasing acidosis - H-hyperionia;
- growing number of K- and Na- ions;
- increasing osmotic pressure;
- expanding enzymatic-autocatalitic processes;
- increasing $CO_2$ in the tissues;
- decreasing surface tension of protoplasm colloids (erythrocytes, leukocytes and histiocytes), which makes the cells stickier, contributes to the phenomenon of “marginal standing” and is followed by changes in the cell configuration.

Inflammatory oedema is formed due to increasing adsorption processes, i.e. binding of water, proteins and salts by the tissues as a result of the growth of osmotic concentration in them; changes of viscosity of issued proteins and the phenomenon of their coagulation when proteins in contact with the denatured tissue surfaces. The basis of the binding of water is the endosmotic and molecular imbibition of structural elements with their swelling and homogenisation (amorphisation), as well as the termination of circulation in the tissue clefts.

At the same time, the inflammatory response has protective and adaptive significance for the organism: proteins of the oedema fluid can bind and immobilise the bacterial toxins, and also neutralise the toxic products of tissue dissolution. This delays the penetration of the above substances from the inflammatory focus into the general circulation and prevents their spread within the organism.

**Thus**, the pathological changes in local tissues during inflammation may be represented as pre-tumour tissue changes.

# 3. FORMATION OF ISOLATED MICRO CAVITIES

***Suess R. et al. (1977):*** *the process of the "birth" of a malignant cell and the development of the malignant process somehow escape from the control of the regulation of growth in the host. This is possible only if the above processes occur in a space which is fairly isolated from the host, but located in its own tissues.*

For the "birth" of a malignant stem cell and the subsequent growth and development of the malignant process, conditions are necessary which isolate the cell precursor from the influence of the organism. These conditions can be described as a "pre-tumour" bed in the pathologically altered local tissues in the background of pre-tumour diseases of the macro-organism.

The "pre-tumour" bed can be an isolated micro cavity, the formation of which is a natural process during inflammation. However, a formed isolated micro cavity will not necessarily become the place where the "birth" of a malignant stem cell will happen.

The emergence of an isolated micro cavity is impossible in epithelial tissue because of the absence of the necessary connective tissue structures, the short life of epithelial cells, as well as their close connection among themselves and with the basement membrane.

The basement membrane plays an important role in the formation of an isolated micro cavity, because it is a reliable barrier guarding the "pre-tumour" bed from external impacts.

The connective tissue is an ideal place for the formation of the "pre-tumour" bed, because its main components: cellular differones, fibrous structures and amorphous substance, having a certain organisation, may participate in forming an isolated micro cavity.

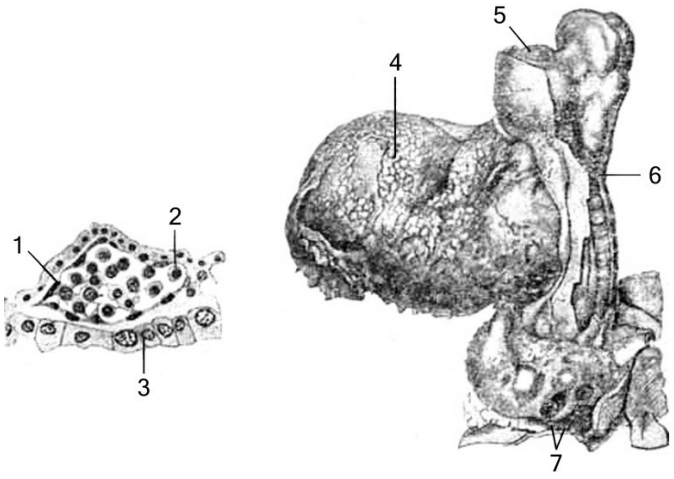
## 3.1 PROTOTYPE

The prototype for the formation of an isolated micro cavity as a “pre-tumour” bed could be the structural organisation of the human embryo’s yolk sac (Fig. 4).

The yolk sac or bladder (Vesicula umbilicalis) of a human embryo is a saccular appendage on the abdominal side of the embryo; it does not have a stock of nutritious yolk. The wall of the yolk sac consists of one germinal layer, its cavity filled with fluid containing protein, and connected with the cavity of the intestines through the yolk duct.

The yolk sac is an auxiliary structure, which ensures nutrition and haematopoiesis of the embryo during the first trimester of pregnancy. Throughout its existence, the yolk sac may grow to 2 - 6 mm in diameter. After 8 - 9 weeks of pregnancy its importance in the development of the embryo begins to decrease and by the end of the first trimester it is completely reduced.

The principle of construction and operation of the yolk sac is the best possible prototype of construction and operation of an isolated micro cavity as the future “pre-tumour” bed, and with this we have:




**A** **B**

Fig. 4. The development of blood islets in the yolk sac of the human embryo (Carnegie Cont. To Emb., 1929, vol. 20):
A - early differentiation of the endothelium and primary blood cells of a 4-week-old foetus (embryo length 4.5 mm): 1 - primary blood cells, 2 - blood cells, 3 - endoderm.

B - embryo at the stage of 10 somites according Koerner: 4 - primordial blood islet in the yolk sac, 5 – cardiac prominence, 6 - amnion’s cut margin, 7 - umbilical vessels.

1. Conditions which emerge inside the micro cavity are similar to the embryonic ones.

2. The “birth” and reproduction of a malignant stem cell initially occur in a closed system isolated from the microenvironment.

3. The malignant stem cell conditionally repeats the beginning of embryonic haematopoiesis.

4. There is a certain amount of nutrients.

5. An aggressive oxygen-free environment is created.

## 3.2 CONDITIONS

1. Congenital and/or acquired formation of pathological cavities and fibrous septa, scars and capsules around the foreign body lead to disturbances in extracellular fluid microcirculation and contribute to the emergence and progress of sluggish chronic inflammation with possible formation of an isolated micro cavity.

2. The destruction of tissues (alteration) facilitates the emergence of lumens in the intercellular space that could turn into a micro cavity.

3. The presence of oedema in the local tissues during inflammation is accompanied by accumulation of fluid (exudate) in some places - in the lumens; some of them with the accumulation of the exudate may transform into a micro cavity.

4. During inflammation there are significant chemical changes in the intercellular fluid, which can be accumulated in a naturally formed micro cavity, thus transforming it into a bulk formation containing aggressive specific fluid.

5. Regenerative processes (proliferation) create opportunities for the formation of the granulation and/or scar tissue that can

develop around the formed micro cavity, turning it into an isolated state with a low content of oxygen or even its absence.

6. There are the pluripotent poorly differentiated cells of the connective tissue series of the peripheral blood in the human body, referred to as “peripheral blood fibrocytes” or fibroblast-like cells, which can migrate to damaged parts of the tissues and participate in the formation of both normal and pathological scarring, as well as an isolated micro cavity.

## 3.3 MECHANISM

***Virchow R. (1863-1867):*** *a chronic irritation of tissues causes their defensive response – the regeneration is going on and the rate of cell division increases, but if the control of the regeneration and the rate of division would be lost, malignant cells may emerge there.*

Chronic inflammation occurs in the background of continuing acute inflammation, while regenerative and inflammatory processes can coexist for a long time, weakened and amplified by one another. At the same time, in the connective tissue, due to the local heterogeneities in the distribution of substances and abnormality of the movement of fluid, it accumulates in the narrow areas of intercellular space.

It is known that there are neutrophils which initially come into the area of inflammation, and which in the focus of inflammation help the organism to clear the area of injury from infection and the decay products of autologous cells, thereby preparing the “work objectives” for monocytes-macrophages. Managing this task, neutrophils sometimes are “too zealous” and generate the “explosion” of destruction in local tissues. So neutrophils can form micro cavities because they cause destruction of the connective tissue matrix, form exudates and attract cells, which provide restoration.

In the process of inflammation the resorption of small blood clots and dead tissues occurs. Large defects of tissue, resulting from fibrinous-necrotic inflammation, are replaced by the scar tissue. Small defects that appear between cells inside the stroma, become lumens in the beginning and then turn into micro cavities.

In response to tissue damage and under the influence of pathogenic factors of inflammation, the pluripotent poorly differentiated cells of the connective tissue series, referred to as “peripheral blood fibrocytes” or fibroblast-like cells, migrate from the bloodstream. Their immunophenotypic signs, combined with the ability to give rise to representatives of the fibroplastic cellular differone, let us suggest that they are a sort of multipotent mesenchymal stomal cells (MMSC), constantly circulating in the blood in small quantities. It is these fibroblast-like cells which are involved in the formation of the insulating coat of a micro cavity.

The final stage is the appearance of infiltration, consisting of small lymphoid cells, which are mainly the product of the reproduction of local connective tissue elements. The cementing substance, covering denatured surfaces of the improvised coat of the emerged micro cavity, is fibrin – a polymerised product of fibrinogen, the blood protein.

It should be noted that the process of chronic inflammation – a long and protracted one, can last months and years, so the formation of an isolated micro cavity can also take months and years.

## 3.4 STRUCTURE

A micro cavity is surrounded by several layers, forming a shell. Each of the layers can appear in a different way and each of them fulfils its role:

**1. The inner layer** - small proliferating fibroblasts, which do not form a continuous mono layer and lie on the middle layer. They synthesize the components of the intercellular substance of proteins (collagen, elastin), proteoglycans and glycoproteins, thus assisting the formation of a connective tissue coat, and also function as a substrate-feeder.

***Laki (1974)****: proliferation of fibroblasts takes place at the fibrin clots, as in the case of wound healing, when fibrin plays the role of a matrix necessary for the regeneration of the tissue structures.*

***Gerasimov I.G., Popandopulo A.G. (2007):*** *fibroblasts, flattened in the mono layer, when deprived of $CO_2$ in the concentration necessary for normal functioning, lose contact with each other, round up and die via necrosis or apoptosis*.

**2. The middle layer** - mainly collagen fibres which surround the micro cavity with a sufficiently dense ring and thus isolate a micro cavity from the influence of the microenvironment.

**3. The outer layer** – erratic, varying in volumes of infiltration, consisting predominantly of small lymphoid cells, which are mainly the product of reproduction of the local connective tissue elements.

***4. O. Meara (1958):*** *spotted threads of fibrin in histological preparations of tumours.*

Fibrin is the result of the increase in the permeability of the vascular wall, the outgoing of blood plasma proteins into the intercellular space and the phenomenon of fibrinogen clotting. Properties of fibrin:
- while in contact with the denatured tissue surfaces surrounding a formed micro cavity, it is a cementing material;
- it influences the growth of a tumour, because it can serve as a food source for malignant cells;
- it protects malignant cells against damaging actions from the host-body, and thereby contributes to malignant growth;
- it prevents the detachment of malignant cells from the main tumour mass;
- it stimulates the processes of acceptance of scattered malignant cells, which give rise to new malignant foci.

## 3.5 SHAPE AND LOCATION

An isolated micro cavity is homogeneously filled with fluid and can be of different shapes: cleft, oval, round, star, etc. (Fig. 5).

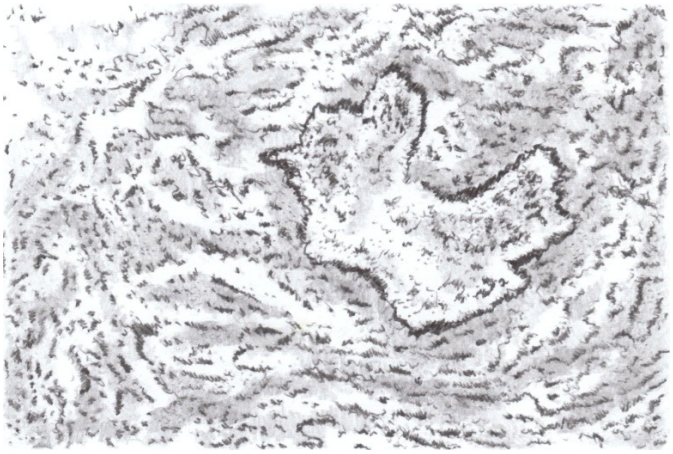


Fig. 5. Isolated micro cavity

The dimensions of the micro cavity depend on:
- the duration and the nature of chronic inflammation;
- the quantity of liquid contained in it;
- the morphological structure of an organ or tissue in which the micro cavity is located;
- the ability of the host-body to restrain the micro cavity.

A primarily formed isolated micro cavity has a size of up to 1 mm, later on its size can rise with the increase of the malignant cells' mass, due to their expansive growth.

The location and depth of lie of an isolated micro cavity depends on many factors, one of them being the shape of the mucous membrane:
- if the shape of the mucous membrane is "flat" (mammary ducts, ducts of the prostate, cervix of the uterus etc.), the micro cavity may be formed under the basement membrane, and possibly the basement membrane takes part in the formation of its coat;
- if the shape of the mucous membrane is "complex" (stomach, large intestine, lungs etc.), the micro cavity is formed deep in the connective tissue at the level of the exocrine glands.

## 3.6 CHEMICAL COMPOSITION OF THE FLUID

The physico-chemical alterations of the intercellular substance in the area of chronic inflammation are significant and manifested in abnormality of the setting of the colloidal systems:
- increase in osmotic pressure (hyperosmia) – similarly in the isolated micro cavity;

- increase in the concentration of hydrogen ions (hyperoxia and acidosis) – similarly in the isolated micro cavity;
- ions of potassium, sodium and chlorine, anions of phosphoric acid and others accumulate due to the destruction of cells - all together or individually they may stay in the isolated micro cavity;
- proteins of the oedema fluid as well as proteins associated with bacterial toxins and toxic products of tissue decomposition may stay in the isolated micro cavity;
- lysosome enzymes (protease, cathepsin, chymotrypsin, alkaline phosphatase etc.) of the cells damaged during the inflammatory process, accumulate in large quantities in the intercellular fluid – may stay in the isolated micro cavity;
- proteins-mediators of inflammation, which have enzymatic features (e.g. necrosin) – may stay in the isolated micro cavity;
- organic acids of the Krebs cycle, fatty acids, lactic acid, polypeptides and amino acids that have emerged in large quantities due to the activity of proteolysis, glycolysis and lipolysis processes – all together or each separately may stay in the isolated micro cavity;
- oxygen quantitatively decreases and carbon dioxide content in the tissues increases, and an oxygen-free environment is created in the isolated micro cavity.

**Thus**, in the area of chronic inflammation, a “pre-tumour” bed is created in the form of an isolated micro cavity, within which the environment is characterised as “super circumstances”.

# RÉSUMÉ

There is “a difficult philosophical question”: an isolated micro cavity – is it a “graveyard” of dead cells or the place of “germination” of immortal malignant cells?

# CHAPTER II

# INITIATION OF THE BONE MARROW MONONUCLEAR CELL

The bone marrow is an organ of the haematopoiesis and at the same time is the central organ of the immune system. The total mass of the bone marrow of an adult is about 2.5-3.0 kg (4.5-4.7% of body weight). About half of this is the red marrow located in the cells of the spongy substance of the flat and short bones, epiphyses of the long (tubular) bones. The rest is the yellow bone marrow, filling the bone cavities of the diaphyses of the long (tubular) bones.

The structure of bone marrow includes stem cells, which form the basic cellular elements of the blood, the cellular elements of the skeletal tissues and the cellular elements of the blood vessels. The younger the person, the more active the bone marrow stem cells are.

# 1. HAEMATOPOIESIS

Haematopoiesis or haemopoiesis - the process of formation, development and maturation of the blood cells in animals and humans. All blood cells originate from a single progenitor cell – a pluripotent haematopoietic stem cell in the embryo genesis, and after the birth; it has the ability to differentiate into all blood cells, without exception, and the ability to participate in the formation of other tissues in the organism. There is embryonic and post embryonic haematopoiesis.

## 1.1 EMBRYONIC HAEMATOPOIESIS

Embryonic haematopoiesis is the process of the blood development, as a kind of tissue, during the embryonic period of human development in the result of three main successive stages:

**1. The first stage** – mesoblastic: in humans it begins at the end of the second/ early in the third week, goes on up to the 9th week of embryonic development, and is characterised by the appearance of **the first generation** of the blood stem cells. In

this case the blood cells appear in the extra-embryonic organs: in mesenchyma of the walls of the yolk sac and in mesenchyma of the chorion and the stem.

Haematopoiesis in the wall of the yolk sac: the rudiments of the vascular blood or blood islets are isolated in the mesenchyma. Inside them the cells become round, lose their appendices and transform into blood stem cells. The cells bordering the blood islets flatten, interconnect, and form the endothelial lining of the future vessel. A part of the stem cells differentiates into the primary blood cells (blasts).

In maintaining the pluripotency of the embryonic stem cells in mammals, the fibroblasts play an important role as a substrate-feeder.

**2. The second stage** - hepatolienal: it begins in the 5-6th week of fetal development when the liver becomes the main organ of the haematopoiesis and **the second generation** of blood stem cells are formed in it. Haematopoiesis in the liver reaches its maximum in 5 months and is completed before birth. The blood stem cells of the liver colonise the thymus, spleen and lymph nodes.

**3. The third stage** - medullar (bone marrow): it begins in the 10th week, gradually increases till birth, and is characterised by the emergence of **the third generation** of blood stem cells in the red bone marrow. After birth the red bone marrow becomes the central organ of the haematopoiesis.

It should be noted that all the organs which carry haematopoiesis: lymph nodes, liver, spleen, thymus and the bone marrow, have certain anatomical and physiological characteristics and share common structural features:
- stroma consists of the reticular tissue, parenchyma – the haematopoietic cells;
- they are all rich in elements of the system of mononuclear phagocytes;
- their capillaries are of a sinusoidal type.

## 1.2 POST EMBRYONIC HAEMATOPOIESIS

Post-embryonic haematopoiesis is the process of physiological regeneration of the blood, which compensates the physiological destruction of the differentiated cells. Normal haematopoiesis is carried out simultaneously by many clones – polyclonally, and all the blood cells are of bone marrow origin.

## The structure of the bone marrow

Bone marrow has certain anatomical and physiological features. Its stroma consists of the reticular tissue, and parenchyma – of the haematopoietic cells. In humans the red bone marrow is a highly vascularised organ; it connects to the bloodstream through the capillary network. There are two types of capillaries: feeding (conventional) and functional (sinusoids), the latter flow into a common trunk - the central vein.
Sinusoids are located in radial order and the haematopoietic tissue is between them. In the sinuses between the endothelial cells there are pores, which connect the tissue of haematopoietic organs with the bloodstream. Such structure provides the cells with the lift to the blood, as well as the inflow of the humoral factors (hemopoietines) from the blood to the haematopoietic organs, which along with the nervous system influence the haematopoiesis. As it is histogenetically single, the blood-forming system is characterised in its functioning by a certain independence of the behaviour of individual shoots of the haematopoiesis.

## The intensity of haematopoiesis

***Vladimirskaya E.B. (2001):*** *a male weighing 70 kg produces daily 1x10 raised to the power of 11 leukocytes and 2x10 raised to the power of 11 red blood cells, which creates a cell mass of 300 grams (100g + 200g respectively) per 24 hours; so per month it produces 9 kg of the cells and per year - about 100 kg. During a life of 70 years the cell production of normal haematopoiesis is very high – about 7 tons of blood cells.*

## Haematopoiesis in the red bone marrow

Haematopoiesis in the red bone marrow takes place in islets, where the cells form groups according to the shoots of haematopoiesis. Precursors and developing haematopoietic cells are located in the following order: in the centre - dividing and

blast cells, in the periphery (near the walls of sinusoids) - more matured cells.

All the blood corpuscles, whose development is extra vascular, are formed from a pluripotent haematopoietic stem cell in the red bone marrow. Some of them are stored in the bone marrow in the undifferentiated state. They can migrate to other organs and tissues and be a source of development of blood cells and connective tissue. Pluripotent stem haematopoietic cells are a self-sustaining population of cells, and they rarely divide.

The stages of bone marrow haematopoiesis constitute six main classes:

**I class - pluripotent haematopoietic stem cell** - the common progenitor cell. Each pluripotent haematopoietic stem cell forms one colony and is called a colony-forming unit (CFU). Research into the cellular composition of colonies has discovered two lines of their differentiation.

**II class** - one line gives rise to a **pluripotent precursor-cell** – the progenitor of the granulocyte, erythrocyte, monocyte and megakaryocyte shoots of the haematopoiesis – and thereby to **the progenitor of myelopoiesis** (CFU-GEMM). The second line gives rise to a pluripotent cell-precursor – the progenitor of lymphopoiesis (CFU-L).

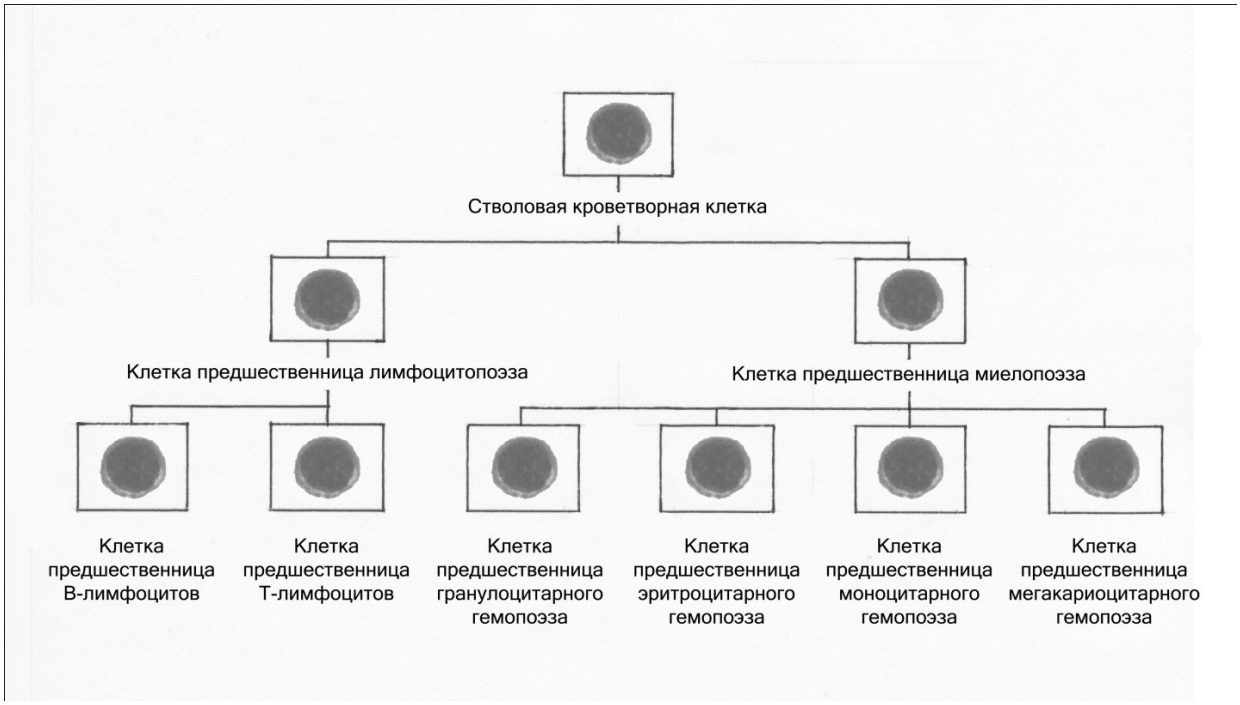



Fig. 6. A general scheme of the first three classes of haematopoiesis:
I class - pluripotent haematopoietic stem cell.
II class - pluripotent precursor-cells of lymphocytopoiesis and myelopoiesis.
III class - unipotent precursor of T- and B-lymphocytes; bi-potent and unipotent precursors of granulocyte, erythrocyte, monocyte and megakaryocyte haematopoiesis.

**III class** - bipotent and unipotent progenitor cells differentiate from the pluripotent cells. The method of colony formation defines **bipotent** and **unipotent precursors of the progenitor of monocytes (**CFU-M), **granulocytes** and **monocytes** (CFU-GM), neutrophil granulocytes (CFU-Gn), eosinophils (CFU-Eo), basophils (CFU-Baso), erythrocytes (BFU-E and CFU-E), megakaryocytes (CFU-Meg), megakaryocytes and erythrocytes (CFU-MegE) and also unipotent progenitor precursor-cells for T- and B-lymphocytes.

**IV class** – bipotent and unipotent progenitor precursor-cells form blast cells of various haematopoiesis lineages, which are identified in the analysis of myelogram (erythroblast, megakaryoblast etc.).

**V class** - maturing cells of various haematopoiesis lineages.

**VI class** - matured cells of various haematopoiesis lineages.

All the cellular elements that are included in the first 3 classes are ontogenetically related and morphologically not differentiated (Fig. 6). Even if attention is drawn to features of the structure of the nucleus and cytoplasm, when studying haematological drugs, they are categorised as lymphoid elements and blast cells. Morphologically recognisable cells are cells of the IV, V and VI classes.

In the process of differentiation the morphologically recognisable cells of the erythrocyte series undergo 5-6 mitoses, and granulocyte cells, 4 mitoses. In the case of monocytopoiesis it takes 7-8 mitoses to pass from monoblast to macrophage.

Cells formed in the red bone marrow upon maturity come into the bloodstream evenly and their circulation time is constant: erythrocytes live 100-120 days and then die, platelets - about 7-10 days and then die, neutrophils - less than 6-8 hours and then die, **monocytes – 1.5 - 4.5 days and then go out into the tissues**.

## 1.3 MICROENVIRONMENT

Myeloid and lymphoid tissues are tissues of the internal environment and are different forms of connective tissue. They represent two main cell lineages - cells of the reticular tissue and haematopoietic cells, i.e. they are characterised by the presence of stromal and haematopoietic elements forming a functional whole.

Reticular cells, as well as fat, mast and osteogenous cells, together with intercellular substance form the microenvironment for haematopoietic elements. The structures of the microenvironment and haematopoietic cells operate in close connection with each other. The microenvironment influences the differentiation of blood cells in contact with their receptors or through the release of specific factors.

## 1.4 REGULATION

Haematopoietic stem cells in the stage of maturation are under strict regulatory control, the mechanism of which has not yet been sufficiently studied. The following play an important role in the regulation of proliferation and differentiation processes of haematopoietic stem cells:

**1. Stromal microenvironment:**
- cellular components: fibroblasts, fat cells, macrophages, osteoblasts and endothelial cells;
- territorial (extracellular) matrix, which consists of the products of the stromal cells secretion: collagen, fibronectin, laminin, glycosaminoglycans and other protein components.

**2. Growth factors** – they provide proliferation and differentiation of haematopoietic stem cells, and subsequent stages of their development. The growth factors include the following:
- colony stimulating factors (CSF) stimulate haematopoiesis. The most studied factors among them are those stimulating the development of granulocytes and macrophages (GM-CSF, G-CSF, M-CSF);
- inhibiting factors – they inhibit haematopoiesis. A leukaemia inhibitory factor (LIF) has been distinguished which inhibits the proliferation and differentiation of monocytes-macrophages;
- interleukines.

**3. Transcription factors** affect the gene expression, determining the forms of differentiation of haematopoietic cells (poietins).

**4. Vitamin B12** is necessary for the stimulation of proliferation and differentiation of the haematopoietic cells.

**Thus**, haematopoiesis in the red bone marrow is the only place in human body tissue where, during embryonic and post embryonic periods of life, a large number of cells of various potencies are concentrated and an intensive proliferation is going on.

# 2. MONONUCLEAR FRACTION IN THE BLOOD SYSTEM

Mononuclear fraction in the blood system comprises the cells released from the bone marrow or peripheral blood through the separation from erythrocytes, platelets and granulocytes by a density gradient.

## 2.1 MONOCYTOPOIESIS

Monocytopoiesis originates from the pluripotent precursor-cell of the progenitor of myelopoiesis followed by the development into a monocyte shoot (II class), and continues with the development into a unipotent precursor-cell of the progenitor of monocytes (III class). However, the cellular elements included in the II and III classes are not differentiable morphologically. Morphological recognition begins with a blast cell of the monocyte shoot (IV class) - monoblast, which turns into a monocyte via the stage of promonocyte.

In contrast to other cell lineages whose cycle of maturation ends in the red bone marrow, the cells of a monocyte shoot finally mature only in the tissues where a promonocyte and monocyte transform into a macrophage.

**Morphology of cells:**

If a monoblast differentiates into a promonocyte and monocyte, the cell undergoes a series of morphological and functional changes (Fig. 7):

C
B
A

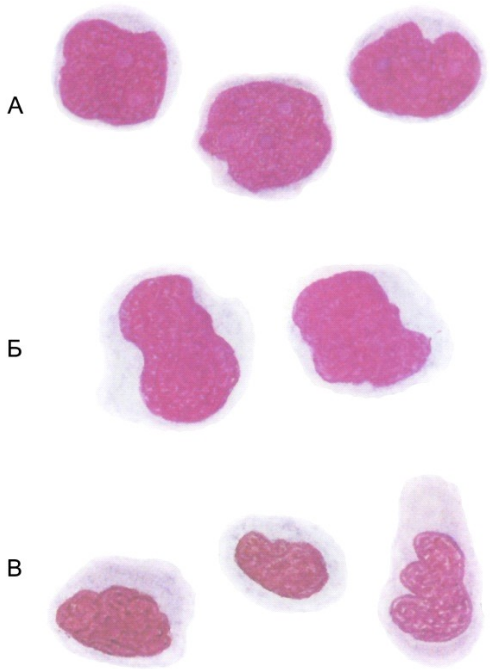



Fig. 7. The lineage of the monocyte cells: A - monoblasts, B – promonocytes, C - monocytes.

**In the red bone marrow**

**1. A monoblast** has a diameter of 12-20 microns. Normally it is difficult to distinguish it from a myeloblast or a non-differentiated blast, and it is also not always possible to distinguish it from a lymphoblast. Only marked outlines of the nucleus and the broader light basophilic cytoplasm may indicate that this “blast” is developing to a monocyte cell. The nucleus of the fine structure contains 1-2 nucleoli of blue colour. The cytoplasm is blue; dust-like azurophilic granules may be present in it.

**2. A promonocyte** has a diameter of 15-20 microns. Normally it has a bean-shaped light violet nucleus of a promyelocyte. The chromatin is fine and wide-meshed. There are 1-2 nucleoli in the nucleus. The cytoplasm is grey-blue and smoke-grey with small azurophil granules.

A promonocyte as a precursor of a monocyte runs through two consecutive cycles of division before turning into a monocyte; the duration of the mitotic cycle is 30 hours. A promonocyte is capable of pinocytosis and phagocytosis, although to a lesser extent than a monocyte or macrophage.

**3. A monocyte** has a diameter of 16-18 microns. The nature and intensity of its nucleus and cytoplasm staining are morphologically various. The nuclei form may be closer to rounded and bean-shaped ones. A monocyte can be categorised as a promonocyte, given the fine structure of its nucleus and the presence of nucleoli (or their remnants). The cytoplasm is grey or pale blue in colour; it may contain numerous dust-like azurophilic granules.

Differentiation of a monoblast into a monocyte occurs in the red bone marrow during a period of 5 days. A monocyte stay in bone marrow averages 3 days (the minimum stay is 9 hours), then it divides and without forming a bone marrow reserve it goes out into the peripheral blood.

## In blood

A monocyte is the largest blood cell. In blood it matures, the nucleus transforming from a round shape to bean-shaped then palmate; and the structure of chromatin changes. A different level of differentiation of monocytes is found in the peripheral blood, with more mature monocytes dominating in the blood of healthy people. There are remnants of nucleoli in an immature monocyte and in the cytoplasm the enzymes change.

In the blood the monocytes split into parietal and circulating pools exchanging with each other, and their quantitative proportions can vary. In humans, in normal conditions the circulating pool of monocytes is of 18x10 raised to the power of 6 cells/kg of body weight, and the marginal pool, which currently does not participate in the circulation, adhering to the inner wall of a microvessel, is 3.5 times bigger (63x10 raised to the power of 6 cells/kg). In general, the aggregate pool of monocytes in the peripheral blood ranges from 1 to 10% of all leukocytes (80-600 x $10^9$ units/litre).

Monocytes circulate in the blood from 36 to 104 hours (1.5 - 4.5 days) and then leave it according to the stochastic (objective) principle, interacting with specialised adhesive molecules in the endothelial cells. The migration of a mononuclear cell from the bloodstream to the focus of inflammation takes place through the micro vascular stream, which has endothelium of the second type – these are post capillaries and venules.

**In tissue**

***Van Furth R. (1988):*** *After getting into the tissue, the blood mononuclear cells transform into macrophages, which in turn adapt to the microenvironment of their future habitation. The heterogeneity of the population of macrophages "horizontally" – is microenvironment in which they operate*.

Coming out of the bloodstream, a blood monocyte becomes a tissue one and is no longer able to return to the circulation. A tissue monocyte transforms into an organ-specific and tissue-specific macrophage according to the following stages of transition: macrophage blast, pro-macrophage, macrophage. A macrophage can be formed from a haematopoietic stem cell and from a promonocyte, according to the same stages of transition.

**4. Macrophage** - a heterogeneous specialised cellular population of the organism's defence system (Fig. 8). Its diameter is 15-80 microns, the shape of the cell is irregular, and the nucleus is oval or oblong. The life expectancy is calculated in months and years.

Macrophages can be formed: in the connective tissue (histiocytes), lung (alveolar macrophages), liver (Kupffer's cells), spleen, lymph nodes, bone tissue (osteoclasts), nervous tissue (microglial cells), skin (Langerhans cells) in pleural effusion and in ascites etc.

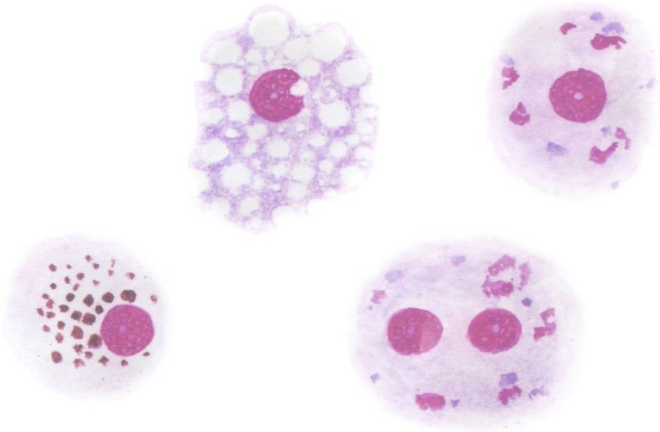


Fig. 8. Macrophages.

Macrophages are active: in non-specific defence against pathogenic micro organisms; in the processes of repair; in initiation of specific immune response; in the metabolism of lipids and iron; in regulation of haematopoiesis; in haemostasis and in the secretion of cytokines and other biologically active substances which regulate proliferation, differentiation and functional activity of different cells.

A variety of phenotypic characteristics of macrophages allows us to imagine the possibilities during the transformation of a promonocyte and monocyte and the influence of the microenvironment. A macrophage by its functional capabilities significantly prevails versus a promonocyte and monocyte. So in the process of transformation, a macrophage acquires the features that were absent in a promonocyte and monocyte.

In pathological conditions human promonocytes, monocytes and macrophages are able to proliferate to a limited extent. A promonocyte of this series is the most proliferating cell and it can, bypassing the monocyte stage, transform into a macrophage in the tissues.

## 2.2 STIMULATION

Given that mononuclear cells are elements of the immunocompetent system, it is quite justifiable to expect a change in their quantitative and qualitative state during various pathological processes. In particular this is evident in the case of chronic inflammation, when the number of monocytes increases. This increase is observed in the red bone marrow, peripheral blood, spleen, lymph nodes and peritoneal cavities. At the same time in the peripheral blood a diverse level of differentiation of monocytes is found, and also the less mature cells - promonocytes may appear and dominate here.

An accelerated version of the proliferation of bone marrow mononuclear cells takes a minimum of 9 hours in the red bone marrow and 12 hours in the bloodstream, totalling 21 hours. For the maturation of a monocyte from a promonocyte two divisions of 30 hours each are necessary, i.e. in the case of urgent demand for macrophages, taking into account the absence of the bone marrow reserve, it is promonocytes and immature monocytes which go into the bloodstream.

In the process of differentiation the morphologically recognisable cells during the monocytopoiesis undergo 7-8 mitoses from monoblast to macrophage. In the case of inflammatory processes the number of macrophages and their activity especially increase. The inflammatory infiltrate can be maintained only in the case of constant renewal of mononuclear phagocytes in the focus, on account of the blood monocytes. In turn, a pool of monocytes is recovered from the red bone marrow. Consequently, the focus of chronic inflammation obtains persistent connections with the bone marrow and with the centre of the fresh monocytes' generation through the mononuclear phagocytes. The focus will progress with the excess of the monocytopoiesis and vice versa. At the same time, activated macrophages take a live part in stimulation of monocytopoiesis.

**Thus**, in the system of haematopoiesis, the promonocyte and monocyte are the only intermediate simplified universal cells, which in the organs and tissues transform into organ-specific and tissue-specific macrophages.

# 3. THE MONONUCLEAR CELL – THE PRECURSOR OF A MALIGNANT CELL

A precursor is a cell which is at a low level of differentiation, but is already committed to develop into cells of a certain lineage.

The axiom of the modern theory of oncogenesis is the notion that the precursor of a malignant stem cell is a normal proliferating somatic cell. However, exactly which somatic cell is the precursor-cell to a malignant cell of a particular solid tumour is not known.

The following very important assertions have been credibly proved and are uncontested:
- malignant cells have more similarity between themselves than normal cells have between themselves;
- malignant cells have fewer differences between themselves than the differences between malignant cells and normal cells;
- normal cells have fewer differences between themselves than the differences between normal cells and malignant cells;

- the basic principles of the “germination” of a malignant cell, of the growth of the primary focus and the development of a malignant process in different organs and tissues are completely identical.

On this basis, we can consider malignant cells as a separate group of cells having common origins and even as a separate tissue in the host-body, if taken in conjunction with the stroma. In such a case it should be a specific cell which claims to be the “common beginning” or the precursor of the primary malignant stem cell of solid tumours.

Analysing all human cells, those having the following properties should be first chosen:

1. They are proliferating somatic cells having a long life cycle (months, years).

2. They are autonomous; they are able to move easily throughout the host-body, penetrate and migrate in organs and tissues.

3. The have an ability to influence various vital processes: haemopoiesis, homeostasis, immunity, proliferation, maturation and differentiation of cells etc.

The only cells in the human body which have the above features are the cells of the blood. Among them:

- Erythrocytes, platelets and white blood cells – these are a dead-locked variant with a short lifetime (100-120 days for erythrocytes, platelets have about 7-10 days and neutrophils have less than 6-8 hours); in addition, they have specific features and quite limited functions, so they cannot claim to be the “common beginning”;

- Lymphocytes belong to the mononuclear fraction of the blood system. They have tropism to the lymphoid tissue and, as is generally known, unipotent and pluripotent stem cells of lymphocytopoiesis are the precursor-cells of the malignant stem cells - haemoblastoses. Mature lymphocytes, when exposed to specific antigens, are again able to transform into blast cells. We can say unequivocally that lymphocytes are directly or indirectly involved in the “birth” of a primary malignant stem cell, as well as in the growth and development of the malignant process;

- Monocytes belong to the mononuclear fraction of the blood system. Their origins are traced to a pluripotent precursor of the progenitor of myelopoiesis followed by its development into a monocyte shoot (II class), which includes quite a large number of cells of different potency (pluripotent, unipotent) and location (bone marrow, vascular bed and the tissues). Therefore, all the cells related to the monocyte shoot are more conveniently called a mononuclear fraction or mononuclear cells. Given its features the **mononuclear cell** is the most likely candidate for the role of the “common beginning” or the precursor of the primary malignant stem cell of solid tumours.

Characteristics and abilities of mononuclear cells (monocyte shoot):

1. Morphologically differentiable and non-differentiable mononuclear cells are categorised into three main groups:

- bone marrow mononuclear cells: a pluripotent precursor of the progenitor of the myelopoiesis followed by its development into a monocyte shoot, a unipotent precursor of the progenitor of monocytes, monoblast, promonocyte, monocyte;
- peripheral blood mononuclear cells: promonocyte, monocyte;
- tissue mononuclear cells: promonocyte, monocyte, macrophage blast, promacrophage, macrophage.

Promonocytes and monocytes are present in all three groups of cells and are an intermediate variant of the development from a bone marrow pluripotent precursor of the progenitor of myelopoiesis, followed by its development into a monocyte shoot (II class), to the organ-specific and tissue-specific macrophage as the final stage of development.

2. Haematopoiesis in the red bone marrow is the only operating focus of intense proliferation, which is extant from the embryonic period of development and is functioning in the adult body.

3. Mononuclear cells represent the cells of the immunocompetent system and at the same time they play a key role in the regulation of normal haematopoiesis. Mononuclear cells can inhibit haematopoiesis through cell interactions and by releasing various immune and non-immune humoral factors.

4. Formation of the cells of the monocyte shoot can occur at any stage of the differentiation from a pluripotent haematopoietic stem cell to the promyelocyte. It is still not clear if there is any difference between monocytes and macrophages, which formed from different subpopulations, and what their specific functions are.

5. The bone marrow mononuclear cells are able to go out of the bone marrow into the peripheral blood, to circulate in the peripheral blood throughout the body, to penetrate from the bloodstream into any organs and tissues and to migrate within them - to travel in the intercellular space.

6. In normal conditions a peripheral blood mononuclear cell matures before it penetrates into the tissue, but in the case of inflammation the period of its stay in the peripheral blood is significantly reduced, so its immature forms, which are capable of active proliferation, penetrate into the tissues.

7. The tissue mononuclear cells are the only cells in the human body which in normal conditions can transform into another blast cell – macrophage blast, followed by differentiation into a macrophage.

8. The peripheral blood mononuclear cell, entering the tissue, does not necessarily transform into a macrophage, it can also turn into a cell of the microenvironment, such as an epithelioid cell (mesenchymal-epithelial transition).

9. Being histogenetically single, the blood-forming system in its functioning is characterised by a certain independence of the individual shoots of haemopoesis behaviour, so *ab incunabulis* the mononuclear cells are characterised by the independence of their behaviour - autonomy.

10. Mononuclear cells retain the ability to divide at all stages of their development and are able to transform themselves into primary malignant stem cells.

11. Malignant cells, like mononuclear cells, have many active features: they influence the proliferation, differentiation and functional activity of various cells; production of growth factors; reproduction in the gel without a substrate; low adhesion; low

contact inhibition; they influence haematopoiesis, the blood-clotting system, cellular and humoral immunity etc.

**Thus,** the tissue mononuclear cells (promonocyte and monocyte), may well claim to be the “common beginning” or the precursor-cell of the primary malignant stem cell of solid tumours.

# 4. GENOTYPIC ALTERATIONS OF A BONE MARROW MONONUCLEAR CELL

A mononuclear cell, as well as any somatic cell, consists of three main components: the cell membrane, cytoplasm and the nucleus (Fig. 9).

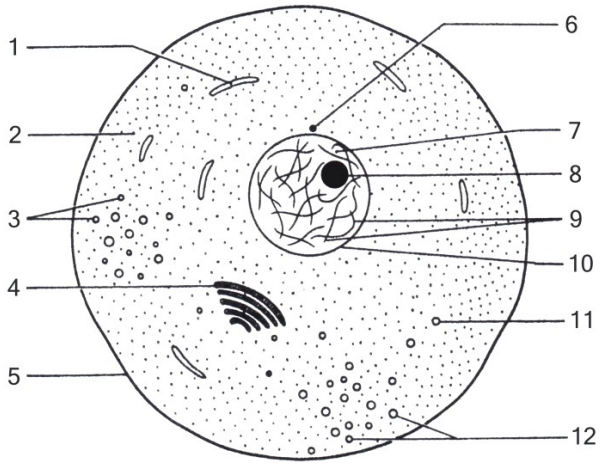



Fig. 9. A general scheme of an animal cell (N. Green, W. Stout, D. Taylor, 1990, Vol. 1, p. 211): 1 - mitochondria, 2 - cytoplasm, 3 - nutrient granules, 4 - Golgi apparatus, 5 - cell membrane 6 - centriole, 7 - karyenchyma, 8 - nucleolus, 9 - chromatin, 10 - nuclear membrane, 11 - lysosomes, 12 - secretory granules.

**Characteristics of a cell nucleus:**

**Nucleus** - the most important structure of a cell, it contains the major bulk of the DNA, which is the carrier of genetic information. In addition, the nucleus regulates the whole “day-to-day” life activity of the cell.

The contents of the nucleus:

1. Chromosomes, which in fact contain all the DNA of the nucleus, are visible as discrete bodies when the cell is at the stage of active division. In the state of rest during the periods between the cell divisions (interphase), chromosomes may not be visible.

2. Nucleolus - a special body, which holds much of the RNA in the resting or interphase nucleus.

3. Nuclear plasma (karyenchyma) - a liquid, which contains salts and proteins but not nucleic acids.

The nucleus is limited to the nuclear membrane (karyolemma), consisting of two lipoprotein layers. The outer membrane is linked with ribosomes, while the inner one closely adjoins the chromatin of karyoplasm. The outer and inner membranes interfuse in the area of the nuclear pores through which proteins and RNA are transported. The pores of the nuclear membrane are filled with protein conglomerate, which isolates karyoplasm from the cytoplasm, so the composition of karyoplasm, including the content of ions, differs from the composition of the cytoplasm.

The gene is a part of the DNA containing the programme of construction of only one specific protein according to the formula “one gene - one protein”. The information contained in the gene is transferred into the cytoplasm through the matrix, or messenger RNA. If the contact of the nucleus to the cytoplasm is terminated, the rate of all reactions in the cell gradually slows down and it dies. During the period of division a “repair” takes place, leading to reproduction and doubling of the DNA molecules, and passing to the daughter cells an identical volume of genetic information in terms of quantity and quality.

## Initiation of the bone marrow mononuclear cell

***Miller E., Miller J. (1966)****: suggested a model of oncogenesis, later named the “theory of molecular-genetic mechanisms of multistage carcinogenesis”. The process occurs in two stages: initiation and promotion.*

We believe that the proposed “theory of molecular-genetic mechanisms of multistage carcinogenesis” in reality covers only a part of the period of “germination” of a malignant stem cell. A model of oncogenesis must include a broader interpretation, as will be expanded on below.

**First stage** (initiation) of the period of a malignant stem cell is “germination”: following the carcinogenic impact (ionising radiation, endo- and exo-carcinogens and viruses) together with the relatively neutral injury of a genome, significant mutations may occur in oncogenes and in anti-oncogenes. As the result of this, a range of distinctive abnormalities occurs at the levels of gene, chromosome and genome: amplifications (increase of gene abundance), deletions, insertions, translocations, micro-mutations (point substitutions, micro-deletions and micro-insertions) etc.

The important provisions of the first stage (initiation) are:

1. Impact of the initiator is primary.
2. The dose of the initiator affects the frequency of the “germination” of malignant cells.
3. Initiation is irreversible.
4. The initiator’s application has to be single and short-term.
5. The initiator acts independently.

It is known that genotypic alterations of the nuclear DNA most often occur in the process of mitosis in the area of active proliferation. In humans, the most intensive proliferation occurs in the red bone marrow, and the most undifferentiated composition of the stem cells approximating to the embryo ones remains here.

The intensity of proliferation in the red bone marrow can increase significantly in the case of a chronic inflammation. This increase is observed in the bone marrow, peripheral blood, spleen, lymph nodes, and peritoneal cavities. With the accelerated proliferation the formation of monocyte series cells can occur at any stage of differentiation from a pluripotent haematopoietic stem cell to a promyelocyte. It is still not clear if there is any difference between monocytes and macrophages which formed from different subpopulations of precursors, and what their specific functions are.

## Genotypic alterations of nuclear DNA

***Boveri T. (1914):*** *the basis of malignancy is a mutation, which occurs under the influence of endogenous and exogenous carcinogenic substances and irradiation.*

In the case of enhanced proliferation with stimulated haematopoiesis, and following the carcinogenic impact (ionising radiation, endo- and exo-carcinogens and viruses), a feature of "genomic instability" may occur in the cell during mitosis.

Along with relatively neutral genome damage, there may be significant mutations in oncogenes and anti-oncogenes. From this an initiated cell emerges, which has specific abnormalities at the levels of gene, chromosome and genome: amplifications (increase of gene abundance), deletions, insertions, translocations, micro-mutations (point substitutions, micro-deletions and micro-insertions) etc.

Genotypic alterations may happen at various stages of the haematopoiesis and, the higher along the scheme of blood formation they occur, the greater potency a malignant stem cell will have. It is therefore important to know not only what genotypic alterations of the nuclear DNA occurred, but at what level of the haematopoiesis they happened, i.e. it is important to understand not only the **character of genotypic alterations** (spectrum of disorders) of the nuclear DNA, but also the **level of genotypic changes** (class in the scheme of haematopoiesis).

As stated above, the stages of the haematopoiesis are divided into six main classes. In each case, following the carcinogenic effects, a variety of genotypic alterations of the nuclear DNA happen, but only in one of the above mentioned classes of the haematopoiesis. For the "germination" of a malignant stem cell of solid tumours the most likely are the following levels of potency:

**1. II class** - a pluripotent precursor-cell of the progenitor of the myelopoiesis, common for 4 shoots (CFU-GEMM) followed by the development to the monocyte shoot.

**2. III class** - unipotent precursor of the progenitor of monocytes (CFU-M).

It has to be emphasised that the genotypic alterations of the nuclear DNA are of a recessive character, so these alterations do not manifest themselves in the red bone marrow in any way. In the process of differentiation, genotypic alterations are input into the genetic apparatus of the future promonocyte and monocyte, which morphologically do not differ from a normal bone marrow mononuclear cell.

Also there is a genetic or hereditary way of transmitting genotypic alterations of the cell's nuclear DNA, where in 1-15% of cases a susceptibility to the development of a malignant disease is transferred to the "blood" relatives. In this case, the genotypic alterations of the nuclear DNA of a bone marrow mononuclear cell have already happened and a carcinogenic impact is not necessary or is just a trigger for their activation.

Acquisition of genotypic alterations by the nuclear DNA is not a decisive factor for a mononuclear cell in its capability to transform into a malignant stem cell. A genotypic-changed mononuclear cell can divide (mitosis), mature, differentiate, transform into cells of the microenvironment and into a macrophage. Although irreversible genotypic alterations of a mononuclear cell are an absolute necessity, they are completely insufficient for its transformation into a malignant stem cell.

The genotypic changes of a mononuclear cell's nuclear DNA will wait patiently until there emerges optimum conditions for their actualisation, and then "conceived" descendants – malignant stem cells - will dominate the host-body. But for the "germination" of a malignant stem cell epigenetic changes are also needed, which can occur only if the cell is in a certain microenvironment, characterised as the "super circumstances".

**Thus,** an initiated cell with various genotypic alterations of the nuclear DNA can emerge in the red bone marrow during accelerated haematopoiesis under a carcinogenic impact.

# RÉSUMÉ

Red bone marrow is the place where the genotypic alterations in the nuclear DNA of a potential precursor of a malignant stem cell of solid tumours emerge.

# CHAPTER III

# "GERMINATION " OF A MALIGNANT CELL

***Rippert V. (1911):*** *in the case of part of the cellular material being freed from the influence of morphological and functional correlations, such cells get full autonomy and they are a structure for malignancy.*

***McKinnell R.G. (1962):*** *proved by experiment the role of epigenetic alterations in the malignant transformation of a cell and their reversibility in principle.*

***Seilar-Asparg (1963):*** *cancer is not a disease of cellular structures, but of a cellular organisation, meaning by this a perversion of some metabolisms which are significant for the cell cycle execution*.

Mechanisms of the cell and tissue determination are within the co-operation of the genetic apparatus of the nucleus with the features of the cell membrane and cytoplasm, which were historically established in the course of phylogenesis and ontogenesis. This means that a mononuclear cell having genotypic alterations of the nuclear DNA will be a potential precursor of a malignant stem cell only if it gets into the “super circumstances” of the microenvironment, which are capable of altering the structure of its cell membrane and the chemical composition of the cytoplasm.

However, for the “germination” of a malignant stem cell it is not sufficient for a precursor to transform into a malignant stem cell, as at the level of the organ’s or tissue’s morphofunctional structures there is competition between normal and malignant

cells, which results in the suppression of the proliferation of some of them, if others are in superior strength.

# 1. MIGRATION OF A TISSUE MONONUCLEAR CELL

Migration of cells is a process of passive dislocation of cells or active movement of cell amalgamations and scattered cells, caused by complex selective interactions of cell receptors located on the membranes of migrating cells with their microenvironment.

Genotypic-changed mononuclear cells (promonocytes and monocytes) from the red bone marrow penetrate into the bloodstream, and from the bloodstream they go into the tissues and then migrate to the area of a chronic inflammation. By chemotaxis some mononuclear cells can get into an isolated micro cavity, because not all of them further transform into a malignant stem cell and the malignant transformation never happens from solitary cells.

There are two variants of how a tissue mononuclear cell (promonocyte, monocyte) can penetrate into an isolated micro cavity:

**The first variant**: the formation of an isolated micro cavity and the existence of genotypic-altered mononuclear cells are separated. At some point genotypic-altered mononuclear cells get inside the almost formed isolated micro cavity. By chemotaxis they seek a formed isolated micro cavity, actively approach it, penetrate through the existing gaps, break the dense part of the shell and find themselves inside the isolated micro cavity as in a “trap”.

**The second variant**: the formation of an isolated micro cavity takes place with genotypic-altered mononuclear cells inside it, which after forming a shell around the micro cavity, find themselves inside as in a “trap”.

It should be noted that in both variants several genotypic-altered mononuclear cells are caught up in an isolated micro cavity, and they are there as in a “trap”. The appearance of epigenetic changes in a genotypic-altered mononuclear cell occurs within the micro cavity only after it has being formed as a system which is isolated from the local tissues.

The conditions in the isolated micro cavity significantly differ from the conditions and the chemical composition of the liquid located in the intercellular space, and these conditions can be described as the “super circumstances”. For example, the oxygen tension in the red bone marrow from where the mononuclear cells came is equal to 40 mm Hg, while in the isolated micro cavity the oxygen tension can be equal to 0-10 mm Hg; i.e. the mononuclear cells can go from the zone of maximum oxygen tension into a zone of severe hypoxia.

In the isolated micro cavity, as in the embryonic environment, there are only mononuclear cells and between them is the intercellular fluid with a specific chemical composition. In this state the mononuclear cells, while in stimulated proliferation are, like embryonic cells, deprived of specialised structures and do not perform special and particular functions. They perform only functions common to all cells: nutrition, respiration, secretion, movement, growth, reproduction etc.

**Thus**, the genotypic altered mononuclear cells get into a micro cavity as into an independent structural entity formed like an embryo’s yolk sac, and find themselves inside as in a “trap”.

# 2. TRANSFORMATION OF A MONONUCLEAR CELL INTO A MALIGNANT CELL

***Shapot V.S. (1975):*** *the most probable cause of neoplastic transformation is a violation of the mechanisms governing the release of genetic information.*

***Mintz B. (1975):*** *Some malignant tumours in mammals may occur as a result of so-called epigenomic changes, i.e. as a result of a steady change in the gene activity similar to that observed in the ontogenesis*.

**Miller E., Miller J. (1966):** *after* ***the first stage****, when irreversible genotypic alterations of the cell's nuclear DNA occur, there may follow* ***the second stage*** *(promotion) of the period of the malignant stem cell "germination": a genotypic-altered cell undergoes the impact of the promoter in conditions which differ from the initial state. First of all, the cell membrane and the cytoplasm are affected.*
The structural alterations of the cell's membrane and the chemical changes in the cell cytoplasm affect the exercise of the genotypic alterations of the nuclear DNA – the epigenetic mechanism.

Important provisions of the second stage (promotion) are as follows:

1. The impact of the promoter is of secondary importance.

2. The dose of the promoter does not affect the frequency of the "germination" of a malignant cell.

3. The promotion is reversible at the early stage of the "germination" of a malignant cell.

4. The promoter's application has to be lasting and continuous.

After a single and short-term carcinogenic impact on the bone marrow mononuclear cell's nuclear DNA, there takes place a prolonged and continuous exposure (months, years) of the cell membrane and the cell cytoplasm to the aggressive oxygen-free environment in an isolated micro cavity in the focus of a chronic inflammation.

## 2.1 STRUCTURAL ALTERATIONS OF THE CELL MEMBRANE

The cell membrane is a functionally active, fine surface structure of molecular scale dimensions, located along the cell surface. The thickness of the membrane is 40 - 60 Å.
**The structure and functions of a cell membrane**

***Singer S.J., Nicolson G. (1972):*** *all cellular and intracellular membranes are composed in the form of the so-called fluid*

*mosaic model; the base of the membrane is a double molecular layer of lipids on which and within which proteins are located. Lipids are the main, isolating and structure-forming component of all biological membranes, while protein molecules are primarily responsible for performing multiple functions inherent in the membranes of living cells*.

Cell membranes are one of the most important structures responsible for the provision of the main vital processes of a cell. The chemical composition of cell membranes depends on their type and function, but the main components are lipids, proteins, carbohydrates and water.

Functions of a cell membrane:

1. Barrier function - separates the living cell from its environment, provides entry for all necessary substances into the cell and the excretion of waste products from the cell.

2. Function of a matrix or a “printed board” – there are proteins and protein ensembles located in a certain order on the membrane; these form a system of electrons transfer, energy storage in the form of adenosine triphosphate (ATP), regulation of intracellular processes by hormones coming from the outside and by intracellular mediators, the recognition of other cells and foreign proteins, the reception of light and mechanical impacts etc.

3. Mechanical function – a flexible and elastic film maintains the integrity of the cell against moderate mechanical loads and disturbances of the osmotic equilibrium between the cell and its environment.

4. The main regulator of cell division:
- the intensity of inflow of substances (ions of calcium and zinc, glucose, nucleosides, chalons etc.), which encourage or block the processes of cell division, into the cell, depends on the state of the cell membrane;
- some substances (peptide hormones, growth factors and possibly chalons) do not penetrate into the cell, but they exert a strong influence on the division process by linking with components of the membrane, thus facilitating or impeding the inflow of other (“trophic”) factors into the cytoplasm and the

nucleus and/or actively affect protein-enzymes affiliated with the membrane;
- hereditarily fixed alterations in the structure, and hence the properties of the membrane, are one of the major causes of uncontrolled growth of tumours.

## Permeability of cell membranes

The permeability of cell membranes is an indicator of the firmness of cells against adverse conditions. The change in membrane permeability is observed from different impacts: in the case of disturbance of water-electrolyte metabolism of cells, drop in temperature, under the impact of metabolic poisons, acidity, hypoxia and anoxia.

The causes of increase in membrane permeability vary:
- change in the ratio of $H^+$ / $Ca^{2+}$ in the membranes (increase of $H^+$ and/or reduction of $Ca^{2+}$);
- decrease in the SH-groups level and an increase of disulfide bonds in the membranes;
- formation of defect areas in the lipids in the membranes, resulting in the accumulation of free fatty acids, lipid peroxidation products;
- increased activity of endogenous phospholipases;
- reduction in the ATP level.

The most universal factor to account for a cell membrane's permeability is the level of concentration of ATP in the cell. In an isolated micro cavity where extreme factors have impacted on the cell, the ATP level decreases; that, in turn, entails the disruption of the membrane functions and limitations in the maintenance of their structure.

It is known that normal somatic cells under the influence of low concentrations of proteolytic enzymes (trypsin) acquire some properties of transformed ones: high agglutinating of lectins, increased accumulation of phosphate and sugar, alteration of the surface charge and significant suppression of contact inhibition. With this, the cell enters a new cycle of mitosis and reproduces, but only by one division.

The chemical composition of plasma and intercellular fluid is similar in normal conditions, but in the presence of an isolated

micro cavity, a chemical imbalance occurs inside it with increasing concentration of one substance and a decrease in others. Thereby the optimal conditions for cells' vital activities are shattered. Accumulation or loss of electrolytes is also reflected in the ion balance of plasma as well as of the whole extracellular fluid. But if it happens in an isolated micro cavity, then the process is limited by this space.

A genotypic-changed mononuclear cell, staying in an isolated micro cavity, is exposed to various aggressive substances in the oxygen-free environment and, in particular, to proteolytic enzymes. The main point of application of these enzymes' impact is the cell membrane. Proteolytic enzymes affecting the cell membrane break its integrity and permeability and also alter its chemical properties. As a result there are structural changes that could alter the selective permeability for inorganic ions. As a consequence, the concentration of low-molecular (inorganic ions) compounds increases in the cytoplasm of a mononuclear cell.

However, alterations in the properties of the cell membrane, such as the content of individual fractions, the charge, electrophoretic mobility, selective permeability and the capacity for agglutination etc. are not the cause of the malignant transformation. Most of these signs are not histogenetically conditioned and can be observed in embryonic cells, regenerating cells and in the cells growing in monolayer cultures.

## 2.2 "CHEMICAL EVOLUTION" IN CYTOPLASM

"Chemical evolution" is a stage of evolution of the cell in which the organic substances acquire new properties in the presence of inorganic molecules and under the influence of external energy and selection factors in the oxygen-free environment, and due to the deployment of self-organising processes, which are common to all relatively complex systems,.

### Characteristics of cell cytoplasm

Cytoplasm of the cell is a semi-liquid mucous colourless mass, containing about 80% water, 10-12% proteins and amino acids, 4-6% carbohydrate, 2-3% fat and lipids, 1% of inorganic and other substances. Cytoplasm consists of hyaloplasm (cytosol), in

which the obligatory cellular components are immersed-organelles and various non-permanent structures (inclusions).

Hyaloplasm or cytosol is a component of cytoplasm, representing its true inner environment. Structurally cytosol is not related to organelles, it contains proteins from which organelles are assembled, and soluble enzymes involved in intermediate metabolism of the cell.

The viscosity of the cytosol varies from liquid (sol) to jelly-like (gel) and increases with a rise in the number of filaments contained therein. Changing the density of the cytosol leads to a change in the relationship of cytoplasm with the nucleus and, as a consequence, the nucleus gets out of control.

Variations in cytosol density:

1. Increase in density – resulting from a reduction of the water content therein or denaturation of proteins.

2. Decrease in density - due to decrease or cessation of protein synthesis, and also the penetration of water into the cytoplasm.

**The mechanism of "chemical evolution"**

***Mekler L.B. (1978):*** *alterations in the permeability of the outer membrane of malignant cells compared with the norm lead to a change in the concentration of inorganic ions within the cell, which also can either change or stabilise the conformation of the protein molecule*.

"Chemical evolution" in the cytoplasm of a genotypic-changed mononuclear cell occurs in the "super circumstances" of an isolated micro cavity:

1. Surrounded by water, which is a chemical compound with a range of properties:
- water is a liquid at the temperature at which the organic molecules are stable, and their synthesis is possible only in aqueous solutions;
- water is required as a depolarising solvent for chemical reactions, because it makes possible a homogeneous mixing and, having a high heat capacity, it takes the heat released during reactions and provides protons for catalysis.

2. In liquids of aggressive chemical composition: a mixture of enzymes, toxins, products of metabolism and cytolysis, inorganic ions etc.

3. In an oxygen-free environment: the chemical synthesis of carbon compounds and their subsequent prebiotic evolution emerge in the cytoplasm. The result is a gradual change of the organic compounds and formation of spatially isolated systems from them.

4. In isolation from the microenvironment: molecules of organic compounds are protected from the influence of the external environment. Delimitation from the external environment and an increased concentration of substances are considered necessary for the formation of biologically active molecules.

5. In the increase in the number of inorganic substances in the fluid surrounding the cell: ions of potassium, sodium, chlorine; anions of phosphoric acid and others. Inorganic substances are often found in bound form, forming compounds with organic substances.

6. In the provision of energy: anaerobic redox reactions in the cells can be a constant source of energy needed for “chemical evolution”.

According to thermodynamics, to perform a useful work it is necessary that at one point the amount of energy or matter was greater than in another; such a difference in the quantity of something between adjacent points is called a gradient. The energy gradient - that is what is necessary for chemical reactions to occur and for the formation of morphological structures.

Given the above, we can conclude that in a mononuclear cell having genotypic-altered nuclear DNA, in the “super circumstances” of an aggressive oxygen-free environment of an isolated micro cavity, the following interrelated processes emerge:

- alterations in the permeability of the cell membrane of a mononuclear cell lead to a change in concentration of inorganic ions in the cell cytoplasm, which are able either to change or stabilise the conformation of the protein molecule;

- organic substances in the presence of inorganic molecules under the influence of external and internal energy and selection factors, acquire new properties; this leads to disruption of the mechanisms governing the release of genetic information;

- overstrain of the redox and other biochemical systems leads to discoordination of making and receiving local, regional and remote signals and it further isolates the mononuclear cell and distorts its signalling and genetic apparatus;

- changes in the chemical composition of the fluid of an isolated micro cavity lead to alterations in the hormonal status; this in turn causes stimulation of proliferation processes and prevents cell differentiation.

The nucleus, as the most important structure of a cell, is not practically affected during changes to the permeability of the cell membrane and the process of “chemical evolution” in the cytoplasm of a mononuclear cell, because the nuclear membrane (karyolemma) reliably isolates the cytoplasm of karyoplasm. Due to this, the composition of karyoplasm, including the content of ions, differs from that of the cytoplasm.

## 2.3 THE MECHANISM OF TRANSFORMATION

The key moment in finishing **third stage** of the period of the “germination” of a malignant stem cell – “the mechanism of transformation itself” - is **mitosis**. It should be emphasised that the **second stage** (promotion) or the stage of epigenetic changes of a mononuclear cell, is not a trigger of its transformation into a malignant stem cell. Moreover, a mononuclear cell which has genotypic and epigenetic alterations, can exist in an unmodified form for an unspecified time, but only until it begins the process of mitosis. And then all its previous changes will reveal themselves. This means the mitosis is a trigger of the transformation, when available genotypic and epigenetic alterations in a tissue mononuclear cell will be revealed in a certain sequence and according to a particular programme.

If mitosis is the starting point for the mechanism for implementing genotypic and epigenetic alterations in a tissue mononuclear cell, so the **basis** of the “germination” of a

malignant stem cell is the law conditioned by the evolutionary development of the human body – a return of any somatic cell to an embryonic state during mitosis.

It is proved that the mitotic state of a cell expresses the passivity of the genetic material, since the physiological and biosynthetic functions of the nucleus and specific activities of the whole tissue cell are blocked. In view of this, the transformation of a mononuclear cell into a malignant stem cell has to be regarded as an abnormality of the mechanisms regulating the gene expression in a changed environment.

**The mechanism of transformation** of a tissue mononuclear cell, which has genotypic and epigenetic alterations, is the **third stage**, which ends the period of the “germination” of a primary malignant stem cell.

Let us recall the following has occurred by this point:

1. In the area of a chronic inflammation the “pre tumour bed" has been formed as an isolated micro cavity.

2. In the red bone marrow, as a result of haematopoiesis and carcinogenic stimulation, various genotypic changes in the nuclear DNA of a pluripotent precursor of the progenitor of myelopoiesis have occurred, followed by the development into a monocyte shoot (II class) or a unipotent precursor of the progenitor of monocytes (III class) according to the recessive sign. The result is an initiated cell with genotypic alterations in the nuclear DNA, but phenotypically it is represented as a normal mononuclear cell (promonocyte, monocyte).

3. Genotype-altered mononuclear cells from the bloodstream have entered into an isolated micro cavity of the chronic inflammation focus, and found themselves as though encased “in a trap”.

4. Under the influence of the “super circumstances” of an isolated micro cavity, structural changes have taken place in the cell membrane of a genotype-altered mononuclear cell, breaching its selective permeability for inorganic ions.

5. Due to the increasing concentration of inorganic ions in the cytoplasm of a genotypic-altered mononuclear cell, “chemical

evolution” has occurred, leading to a breach of the mechanisms regulating the gene expression.

6. As a result of structural alterations in the cell membrane and chemical changes in the cytoplasm of a genotypic-altered mononuclear cell, the process of epigenetic changes has been completed.

Each of the above processes - acquisition of genotypic and epigenetic alterations - is important in the preparation of a tissue mononuclear cell for the final mechanism of transformation: the “germination” of a malignant stem cell. Moreover, the mechanism of transformation can occur and be successfully completed only if a sufficient number of mononuclear cells be concentrated in the isolated micro cavity, because most of them die during the transformation process.

It is known that in tissues in the process of transformation every promonocyte and monocyte turns into a macrophage. The transformation is a series of cell divisions, and with this the phenotypic alterations in cells occur consecutively under the influence of the microenvironment. During each cell division (mitosis) a return of the mother cell to the embryonic state happens as an evolutionary law.

**The third stage – the mechanism of transformation** of a tissue mononuclear cell, which has genotypic and epigenetic alterations, into a primary malignant stem cell should be considered as a continuous process, consisting of two parts:

**Part one** (emergence of the level of genotypic alterations):
- developments leading to mitosis: increasing size of the nucleus and the cell in relation to their swelling; the cell becomes round, its organelles disappear etc.;
- in the process of mitosis, there is a return to an embryonic state: the cell simplifies, loses signs of specialisation and the first phases of mitosis are accompanied by increased permeability of the cell;
- after the mitosis: due to occurred epigenetic alterations it launches the process blocking the differentiation of daughter cells.

***Morgan T.H. (1934):*** *the problem of differentiation is essentially a problem of the differential activity of genes.*

***Spigelman S. (1948):*** *the differentiation and the mechanism of gene activity - two sides of the same phenomenon.*

***Potter V.R. (1969):*** *oncogenesis is a blocked ontogenesis, if the word "blocked" is understood as an abnormal stop to development, breaching the co-ordination of the gene expression.*

***Truman, D. (1976):*** *cancer can be considered as a manifestation of pathological cellular differentiation.*

***Goldstein J.L. (1980):*** *cancer is a disease of the mechanisms regulating the gene expression*.

The level of the block of differentiation corresponds to the level at which the genotypic alterations in a bone marrow cell occurred during haematopoiesis: a pluripotent precursor-cell of the progenitor of myelopoiesis with subsequent development into a monocyte shoot (II class), or a unipotent precursor-cell of the progenitor of monocytes (III class). We can say that the level at which the genotypic alterations of a bone marrow cell's nuclear DNA occurred is an obstacle to the transformation of a tissue mononuclear cell into a macrophage.

So in conditions close to the embryonic ones, surrounded by an aggressive oxygen-free environment, the emerged undifferentiated daughter cells get in very difficult circumstances: they cannot return to the initial phenotypic state of their mother cell - promonocyte or monocyte - and they are not capable of transforming into a macrophage blast with subsequent differentiation into a normal somatic cell – a macrophage.

**Part Two** (manifestation of the nature of genotypic changes): the genotypic alterations in a cell's nuclear DNA take effect, i.e. the spectrum of abnormalities appears at the levels of the gene, chromosome and genome: amplifications (increase of gene abundance), deletions, insertions, translocations, micro-mutations (point substitutions, micro-deletions and micro-insertions) etc.

The nature of genotypic changes indicates to what level the genes have had exposure in relation to the initial embryonic

cells, so that the ability to influence them appears followed by subsequent manifestation of the embryonic and hormonal properties of a malignant cell.

The genotypic alterations of the nuclear DNA of a promonocyte or monocyte disturb the normal function of genes and proteins coded by them. The result is a gradual transformation of the emerged undifferentiated daughter cell into a variant of a malignant stem cell, and with this:
- the number of asperities on its outer membrane increases;
- the microvilli are formed, which increase the ability of the cell to firmly adhere to a foreign surface;
- the nuclear-cytoplasmic ratio increases.

As a result, an unstable active system “germinates”- a primary malignant stem cell, which retains many properties of the mother cell, the tissue mononuclear cell (promonocyte, monocyte), but has not fully left the embryonic state and acquired the capabilities of its new life:
- invasive growth;
- strengthening of autonomy;
- a change in energy production from aerobic to anaerobic;
- the emergence of embryonic signs;
- uncontrolled division and proliferation;
- immortality of the cell population.

The “germination” of a malignant stem cell is gradual or saltatory during a few cell cycles, thus creating the possibility to select cells having more expressed malignancy, and therefore during differentiation, morphological and functional changes initially appear, and then the ability for uninterrupted division.

As a result of the proliferation of a “germinated” malignant stem cell in the space of an isolated micro cavity, the **first generation** of malignant stem cells emerges. A feature of this cluster of malignant cells is that all of them are clones. The change in their qualitative properties is conditioned by the genotypic instability and aggressive influence of the environment in a confined space.

A malignant stem cell is a proliferating somatic cell that has a certain level of potency, which corresponds to the level at which the genotypic alterations occurred in the bone marrow cell during haematopoiesis:

1. If the genotypic alterations in the bone marrow cell occurred at the level of a pluripotent precursor-cell of the progenitor of myelopoiesis with the following development into a monocytic shoot (II class), the tissue mononuclear cell transforms into a pluripotent malignant stem cell, which has an expressed phenotypic heterogeneity and possible emergence of a “cell-chimera” with multiple differentiation.

2. If the genotypic alterations in a bone marrow cell occurred at the level of a unipotent precursor-cell of the progenitor of monocytes (III class), the tissue mononuclear cell transforms into a unipotent malignant stem cell, which has minimal phenotypic heterogeneity.

A malignant cell with the above capabilities can be called the “true malignant cell”. In contrast, later on another cell may emerge, which can be called a “conditionally malignant cell”. The latter is a result of the phenotypic alterations of cells of the epithelium (tectorial or glandular) under the influence of the growth factors released by the true malignant cells into intercellular space.

**Thus** the “germination” of a malignant stem cell is the result of successive genotypic and epigenetic alterations of the tissue mononuclear cell, which occur during its mitosis in the “super circumstances” of the isolated micro cavity’s microenvironment.

# 3. INFLUENCE OF THE MICROENVIRONMENT ON THE “GERMINATION” OF A MALIGNANT CELL

The phenotype of normal, pre-tumour and malignant cells can vary and such alterations dependent on the state of cell contacts and on the characteristics of the cell microenvironment. The microenvironment is a system directly affecting the cell components of its local environment.

**The red bone marrow**

Polymorphism of malignant cells is conditioned by two variants of the transformation mechanism:

- **one option:** the red bone marrow supplies an area of chronic inflammation with mononuclear cells, which are similar in the level and nature of their genotypic alterations. As a result of a tissue mononuclear cell's transformation, the "germination" of a malignant cell of a particular phenotype occurs. However, under the influence of an aggressive oxygen-free environment during the process of differentiation, phenotypic variants of malignant cells may develop. As a result, the aggregate pool may consist of phenotypically different malignant cells, thus creating polymorphism;

- a**nother option**: the red bone marrow supplies an area of chronic inflammation with mononuclear cells differing in the level and/or the nature of their genotypic alterations. As a result of a tissue mononuclear cell's transformation, the "germination" of a malignant cell of different phenotypes occurs. Under the influence of an aggressive oxygen-free environment during the process of differentiation, these variations will manifest themselves, and a range of phenotypic variants of malignant cells will emerge. As a result, the aggregate pool may also consist of phenotypically different malignant cells, creating a polymorphism.

## The bloodstream and the tissues

In the bloodstream and the tissues, a genotypic-altered mononuclear cell is under the influence of the microenvironment from its egression from the bloodstream during migration until its penetration into an isolated micro cavity, and this may affect its phenotype status. A mononuclear cell can take features of the tectorial or glandular epithelium – the mesenchymal-epithelial transition. After the transformation, these changes may manifest themselves in the malignant stem cell and, if so, signs of the tectorial or glandular epithelium will be observed in it.

## Isolated micro cavity

Unequivocally, the greatest influence on the subsequent phenotype of the "germinating" malignant stem cell is exercised by the structures located inside the micro cavity:

**1. Substrate**: in the micro cavity, a mononuclear cell and then the malignant cells affix to the surface of the collagen fibres of the coat or form around themselves an extracellular matrix of proteins and glycosaminoglycans.

**2. The stromal microenvironment:**
- cellular components. These are the cells to which a mononuclear cell and then a malignant cell contacts: fibroblasts, lymphocytes, tectorial and glandular epithelium, connective tissue cells and others. Fibroblasts have an organising influence on the culture of the “germinated” malignant cell by determining its orientation and orderly organisation . At the same time a malignant cell stimulates proliferation of fibroblasts by releasing substances which assist the growth of the fibroblast culture;
- extracellular matrix, which is composed of the products of stromal cells’ secretion: collagen, fibronectin, laminin, glycosaminoglycans and other protein components.

**3. The humoral environment**: in the first place, it is a liquid contained inside the isolated micro cavity; in the second, the intercellular fluid, blood and lymph.

All three forms of the microenvironment - substrate, the stromal microenvironment and the humoral environment, control morphology (shape and position of the cell), reproduction and differentiation of the cell.

**4. Growth factors:** “germinated” malignant cells move on to autocrine and paracrine mechanisms of their growth regulation, which ensure proliferation, differentiation and subsequent stages of their development.

Heterogeneity of malignant cell populations: there is a predominance of Monoclonal solid tumours (80%) over the polyclonal ones (20%).

However, conditions in the isolated micro cavity just contribute to the “germination” of a primary malignant stem cell, which is not the final kind of cell in the process of malignant progression and natural cellular selection. After the emergence of early forms of malignant cells, the isolation of a micro cavity gradually becomes conditional, since the need for nutrients, water and oxygen begins to prevail. As a result, the insulating coat around a micro cavity becomes permeable and the influence of the

external environment of the micro cavity on the mechanism of malignant progression increases.

**Thus** the microenvironment influences at all stages of the "germination" of a malignant stem cell: in the red bone marrow, the bloodstream, tissues and the isolated micro cavity.

# 4. A MALIGNANT CELL

***Davydovskiy I.V. (1962):*** *tumour cells are normal cells, but in specific conditions of existence.*
***Shapot V.S. (1975):*** *the specific properties of a cancerous cell are generated by the same elements as in a normal cell*.

A malignant cell, like any other cell, is an elementary structural, functional and genetic unit of the human organism (Fig. 10, 11).

As a living system, the cell maintains and restores its integrity, adapts to its environmental conditions, grows and develops at the expense of energy and substances that are replenished from its surroundings. It releases the end products of metabolism and part of the energy produced into the external environment. A malignant cell breathes, eats, feels, moves, works, reproduces, remembers its past state and prepares for future challenges.

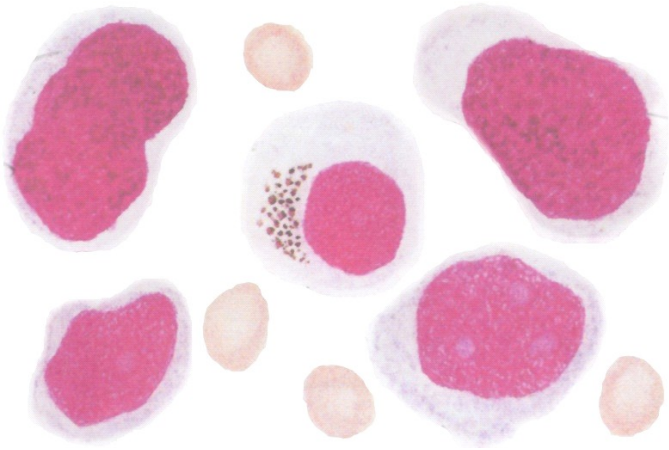


Fig. 10. A liver cancer (cytology): there is a normal cell (hepatocyte) in the middle of the agglomeration of malignant cells.

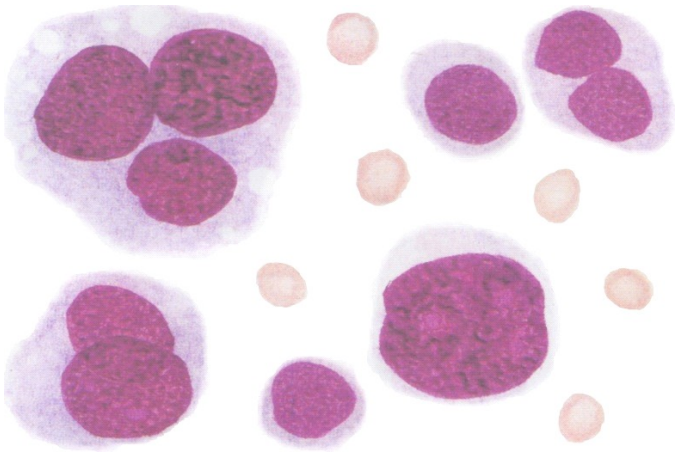


Fig. 11. A kidney cancer (cytology): there is a normal cell (epithelium of tubules) in the bottom-middle of the agglomeration of malignant cells.

## 4.1 DIFFERENTIATION AND MATURATION

***Strong E.L. (1961):*** *cancer is an intermediate stage of some process or the reverse development of the organism from simple to complex*.

Differentiation - the emergence of differences in initially identical cells during the development process, leading to their

specialisation. Differences between cells are defined by a set of proteins synthesised in them. An undifferentiated cell is a cell that lacks the characteristics of this or that tissue origin and is capable of undergoing the process of differentiation.

Maturation is a differentiation of cells and formation of key enzyme systems. The cell is preparing to perform the functions intended by nature, through gradually activating its metabolism. Mature cells are cells that end the histological series.

A “germinated” malignant cell, as well as all normal somatic cells, is exposed to the process of maturation and differentiation. The differentiation of cells in malignant tumours is not mainly retained, as it comes from the newly formed undifferentiated daughter cells.

Under the influence of many of the above factors, malignant cells of varying degrees of maturity and level of differentiation emerge. It is therefore appropriate to speak not about how far a malignant cell has moved off or approached to a “mythical” normal somatic cell, but about what degree of maturity and what level of differentiation a malignant cell has obtained during the process of its development, compared with the malignant stem cells of the related generation.
In fact, we can speak about the organisation of a new differone. A differone (histogenetic series) is a set of cell shapes that forms a particular line of differentiation. In composition, malignant cells are consecutively distinguished into the following: stem cells, precursor-cells and cells of various degrees of maturity and levels of differentiation.

Evolution of the malignant cell has its own peculiarities. In the early stages of oncogenesis and during progression, malignant cells undergo significant changes in their characteristics. The tendency of these changes is to strengthen the signs of autonomy and malignancy. Later there is only a relative stability of all characteristics observed in the cell population of a malignant focus.

In fact, the changes are much slower and the material for evolution is no longer rough alterations of karyotype and other cellular structures, but more subtle and difficult to detect features of the structure and the organisation of the cell’s hereditary apparatus. This means that any malignant cell is a

temporary interim version of the development of the population. Granted the immortality of the malignant cells' population, it is impossible to see the final version of the development.

As a result of differentiation a malignant cell can change its shape, ultrastructure and type of behaviour; with this, the organelles can be lost or acquired and some functions can appear or disappear. Sometimes different types of malignant cells synthesise specific substances, but often they differ in a characteristic set of substances which are common to many cell types, but present here in certain concentrations.

Given that the precursor cell for a malignant stem cell is the tissue mononuclear cell, which initially is phenotypically different from the parenchymatous cells of the microenvironment, a phenotypic homogeneity of malignant cells and the cells of the microenvironment cannot be expected. The degree of maturity and level of differentiation of malignant cells are to be considered in the context of their emergence, growth and development, but not in comparison with that of the cells of the microenvironment.

The precursors of malignant cells of various degrees of maturity and levels of differentiation are the malignant stem cells, in which the processes of specific syntheses have either terminated or are negligible, and which are primarily responsible for restoring and increasing the number of malignant cells during the growth of tumours. Therefore, the dynamics of the change process in the signs of maturity and differentiation can be represented as follows:

- from an immature and undifferentiated malignant stem cell to an immature and an undifferentiated malignant cell of different phenotypic orientation;

- from an immature and undifferentiated malignant stem cell to a malignant cell of different maturity and level of differentiation.

The selection of malignant cells is made mainly according to the phenotype, so the phenotypic heterogeneity of the population of malignant cells can be seen as the only phenomenon of the natural selection in the multi-cellular organism; a result of this is the emergence of cells which dominate over separate malignant cells. Less differentiated and therefore more malignant cells

have a greater potential for progression, and force the less malignant ones out of the population. However, because of certain characteristics of an organism or under the influence of certain factors reducing variability and malignancy, separate clones having low malignancy may acquire selective advantage and force out of the population the more malignant variants, as the latter have a slowdown in the rate of growth.

## 4.2 GENERAL DESCRIPTION

Reproducibility of a malignant focus by a single cell means that each individual cell is hereditarily changed and is a carrier of a “malignancy” sign. At the same time, an individual malignant cell does not possess any signs of malignant growth: the ability to invade, metastatic activity, stimulation of stroma formation and systemic impact on the organism etc. All this can occur only in the offspring, when a malignant cell reproduces a cell population with a malignant phenotype.

Malignant cells successfully compete with normal cells because they are longer lived, they reproduce themselves faster and successfully compete for vital nutrients, they can penetrate the nearby tissues and destroy them, they can also spread themselves through blood and lymph within the host-body and are able to organise the secondary foci - metastases.

**Main features:**
- heterogeneity of the population;
- polymorphism and atypical populations;
- increased ratio of “cytoplasm/nucleus”;
- high level of the cytoplasm’s RNA;
- the presence of numerous mitoses occurring with abnormalities;
- disturbance of the genetic material distribution;
- increased proteolytic activity.

**Ultrastructure**:
- increased segmentation of the nucleus;
- increasing quantity of functionally active euchromatin;
- presence of intranuclear inclusions following the organelles’ entry during mitosis;
- mitochondria are small in number, fragile and polymorphic;
- Golgi apparatus is poorly developed;

- rough endoplasmic reticulum is poorly developed.

## 4.3 FEATURES

### Autonomy

From the beginning, a mononuclear cell, like the precursor-cell of a malignant stem cell, has autonomy. The transition from a normal to a genotypic-altered cell and finally, to the malignant stem cell, occurs in successive stages, each of them requiring a certain period of time measured in months or even years. This helps strengthen the autonomy of the cell. The “germination” of a primary malignant stem cell and its subsequent proliferation occur in an isolated micro cavity, thus escaping the influence of the microenvironment of the host-organism and also helping to strengthen the autonomy of the malignant cells.

### Immortality

In cytology, there are concepts of a “cell’s life span” and a “cell population’s life span”. In the case of a malignant cell the concept of “immortality” is applied, but only in relation to the “cell population’s life span”.

In most cases, malignant cells live much less than most human somatic cells. The explanation of this may lie in the following:
- high level of variability - genotypic and epigenetic instability;
- low adaptability to the conditions of existence in the organism compared with normal cells;
- in some cases their selective value is inferior to the selective value of somatic cells of the microenvironment, which are in the organism under the influence of the stabilising selection for those having the normal phenotype.

### Reduction of “contact inhibition”

***Holley W.R. (1975):*** *contact inhibition is a proper control mechanism, which could best be described as a “regulation which depends on the cell density”*.

Normal cells grow to a maximum density of $10^4$ units/$cm^2$, malignant cells up to $10^6$ units/$cm^2$. Disturbance of the phenomenon of the malignant cells’ “contact inhibition” is caused by an increase in the density of cytosol, which is a

component of cytoplasm, representing its true inner environment.

## Reduced adhesion

***Aub (1963):*** *Malignant cells' adhesion is weaker, and they more easily agglutinate under the affect of lectins than do normal cells*.

Adhesion is the ability of cells to bind selectively to each other or to components of the extracellular matrix. During histogenesis, cell adhesion controls the beginning, the progress and the finish of cells' migration and the formation of cellular communities.

The reduced adhesion of malignant cells compared with normal cells of the microenvironment can be explained by the fact that malignant cells have descended from blood cells - mononuclear cells, which are not adhesive in the normal state. Tissue promonocytes and monocytes also retain non-adhesiveness and have the ability to move actively in the intercellular space.

## Mimicry

Mimicry is a protective instrument, which in nature most frequently occurs in insects.

In this case, we are talking about the mimicry of malignant cells as an instrument designed for protection or for aggression, providing them with partial morphological and/or histochemical resemblance to normal somatic cells of the microenvironment.

In the period of formation of the primary focus, the mimicry of malignant cells has a protective function against humoral and cellular immunity. Then, as a result of progression, the malignant cells take on aggressive features according to the principle: the lower the level of differentiation and maturity, the more aggressive the cell.

The mimicry of malignant cells is consistent with all the principles that exist in biology:

1. Malignant imitator-cells are found together with the original models (somatic cells of the microenvironment).

2. In the initial stage of their development, malignant imitator-cells are more vulnerable and less stable than somatic cells of the microenvironment.

3. In quantitative proportion, malignant imitator-cells are in a significant minority against the original models.

4. There is no complete coincidence of morphological and histochemical characteristics of malignant imitator-cells and the original models.

5. Malignant imitator-cells show their ability at the time of "germination" and the growth of the primary focus, when there is the need for their quantitative increase, in order to achieve the critical mass which is a vital necessity and the basis for survival.

**Anaerobic glycolysis**

***Warburg O. (1956):*** *malignant cells arise through selection in the case of abnormalities of the normal cells' respiration process. Most cells die in such conditions, but those which during the selection process have changed their metabolism for intensive glycolysis, i.e. oxygen free energy release – survive, reproduce and make a malignant focus in a number of generations.*

Warburg was absolutely right. Indeed, in the connective tissue the low content of oxygen in the area of chronic inflammation and lack of oxygen in the isolated micro cavity mean a virtually oxygen-free environment. In these circumstances, in the process of mitosis a transformation of the genotypic- and epigenetic-altered mononuclear cell into a malignant stem cell can occur. Most cells die in these conditions, but the one that has successfully transformed into a primary malignant stem cell acquires the predominance of energy supply from anaerobic glycolysis.

**Embryonic characteristics**

***Cohnheim J.F. (1875):*** *in an early embryonic period a large number of cells are produced, from which a cell mass can potentially arise. They settle in the body and under adverse conditions (trauma, immunosuppression, prolonged mechanical stimulation) can give rise to a malignant growth*.

Numerous studies on the reactivity and the histogenesis of tissues indicate that no embryonic rudiments-foci or cell masses, which would be blocked at the level of embryogenesis, exist in the postnatal period. Moreover, alteration of the isoenzyme spectrum of enzymes towards the embryonic spectrum, as well as the synthesis of embryonic antigens, occurs during the regeneration, inflammation, neurogenic dystrophies and chronic hypoxia of different origin.

Still Cohnheim was right, but it is not the embryonic rudiments-foci but the conditions which are similar to embryonic. A genotypic-altered mononuclear cell gets into these conditions and there acquires additional epigenetic alterations. Then there is mitosis, during which the mononuclear cell returns to the embryonic state, like any normal proliferating somatic cell. With this, mitosis functions as a trigger when the cell's acquired genotypic and epigenetic alterations manifest in a certain sequence and according a certain programme. After mitosis the block of differentiation and the transformation of a tissue mononuclear cell into a primary malignant stem cell emerges. Therefore, in the case of the malignant process the embryonic features are characterised by the more (critical) depth of the "return"; they are stable and inherited in a number of subsequent cell generations.

## Malignant progression

***Foulds L. (1969):*** *the tumour is considered as a mass, continually progressing through qualitatively different stages, which refer to hereditary alterations of an irreversible nature of one or more clearly distinct signs*.

***Nowell (1974):*** *the clone evolution, underlying the tumour progression, is a search by the damaged cell for a new equilibrium, and this process cannot be accidental*.

After "germination", the genotypic and phenotypic heterogeneity of a malignant cell continues with the presence of genotypic/phenotypic instability. In the process of tumour development, this is a constant source of increasing phenotypic diversity of malignant cells and it creates conditions for the permanent selection of variants which are the most adapted to the environmental conditions.

It should be noted that malignant progression is not an objective in itself. The defining conditions for the transition of malignant cells from one qualitative state to another or from one generation of malignant stem cells to another, change the place of malignant cells' localisation and the specifics of the microenvironment's influence. In this case the principle of extension in time and space takes effect.

**Thus,** polymorphism of malignant cells is due to a variety of possibilities for their "germination", maturation and differentiation, as well as evolution and the influence of the microenvironment.

## RÉSUMÉ

The mononuclear cell - the cell which is dedicated to protecting the host-body - transforms into a "killer-cell".

# CHAPTER IV

# GROWTH OF THE MALIGNANT FOCUS

***Bohmig (1937):*** *malignant growth is not a wild, chaotic, unorganised and atypical growth, but orderly growth having its determinate differentiation, which differs only by unusually high intensity*.

***Kavetskiy R.E.(1938):*** *the basis of carcinogenesis is the local process of a tumour germ formation and general alterations in the body, causing the possibility of the transformation of a tumour germ into a real tumour*.

# 1. THE MALIGNANT “GERM”

A germ is an organism in the formative period of its definitive structure’s basic features; it is located inside the chorions or in the body of the uterus.

After its “germination”, the malignant stem cell actively proliferates, followed by the formation of similar or homogeneous malignant cells, which are located within the coat of an isolated micro cavity. In this way, the clone of cells specific for this malignant neoplasm is formed.

The ability of malignant cells to grow in semi-liquid media contributes to the fact that they lose the need for close connection with the substrate: so in the micro cavity one part of the cells sticks to the inner surface of the coat, the other is in free state or in suspension.

With the growing impact of the microenvironment, as well as the presence of the unstable genetic apparatus of the primary malignant stem cell, the **first generation** of malignant stem cells appears.

The first generation of malignant stem cells has a complete set of potentially malignant properties: activated oncogenes, minimal differentiation, clonogenic capabilities, immortality and autonomous regulation. With the accumulation of the mass of malignant cells inside the micro cavity, a lump rises, which may be conditionally called a malignant “germ”. The emergence of this “germ” is due to various pathological processes of local character and general alterations in the body.
At first the malignant “germ” is composed of a small group of malignant cells, whose nutrition is provided by the protein reserves contained in the micro cavity, dead cells, fibroblasts and fibrin, as well as through one-way diffusion of nutrients, water and oxygen from the intercellular space.

The result of active proliferation is a gradual increase in the number of malignant cells; the nutrition of the malignant “germ” is already provided mainly by the inflow of nutrients, water and oxygen from the intercellular space. The need to remove the products of cell metabolism helps the isolation of the micro

cavity from the external environment to become more and more relative.

At the beginning, the malignant “germ” has a size of up to 1 mm, further on it can significantly increase as a result of expansive growth. In this form and at such size the malignant “germ” can exist indefinitely. It depends on many factors, including the following:
- the level of malignancy of malignant cells;
- the ability of a micro cavity’s coat to keep the clone of malignant cells within its borders;
- the capability of the host-body to securely isolate the micro cavity from the surrounding tissues;
- the capability of the host-body to effectively kill the “germinated” malignant cell and its evolving clone.

The outcome of the development of a malignant “germ” may be the following:

1. Spontaneous regression, due to its successful isolation and the active influence of the host-body’s immune system effectors.

2. “Emigration” to the basement membrane, with the formation of “carcinoma in situ”.

3. An increase in size up to the dimensions of the exocrine glands surrounding the micro cavity, with the imitation of their structure and functioning.

4. Full or partial destruction of the micro cavity’s coat and the outgoing of malignant cells into the intercellular space, followed by formation of the primary focus of the malignant process.

**Thus**, a malignant “germ” is a clone of malignant cells inside the isolated micro cavity in the formative period of the basic features of the primary malignant focus.

# 2. THE PRIMARY FOCUS

A further increase in the number of malignant cells, total or partial destruction of the micro cavity’s coat, the outgoing of

malignant cells into the intercellular space with the involvement of the stroma of an organ or a tissue in the malignant process, mark the beginning of the primary focus of a malignant process.

Involvement of blood vessels in the process allows the malignant cells to receive nutrition and oxygen directly from the circulating blood. With this, colonies of malignant cells have the opportunity to further growth and development, that manifests itself by the relatively rapid increase in the size of a malignant focus and the appearance of clinical signs of the presence of a malignant process.

The general rule for the primary focus is a well-known constancy of its individual structure for the entire period of growth and development. The specific morphological features of this form of a malignant neoplasm have mostly been determined already in the malignant “germ”. However, there are numerous exceptions to the rule, with the evolution of the structure of malignant neoplasm either towards a more differentiated or (more often) towards a less differentiated structure.

## 2.1 FEATURES

***Cowdrey (1955):*** *in most cases, the cancer tumour is a tissue or a combination of tissues, and not just an agglomeration of altered cells*.

A malignant neoplasm, like any normal tissue, is composed of stroma and parenchyma, with a different qualitative and quantitative ratio of stroma and parenchyma.

### Stroma

***Bogomolets I.I. (1956): the*** *stroma is not an inert skeleton, but the soil on which the body’s ability to develop greatly depends. The condition of the stroma largely determines the likelihood of development of typical pathological processes in the body: inflammation, necrosis and tumour*.

The stroma is an important structural component of a malignant neoplasm. None of the tumour can exist without the stromal component which, on the one hand, is the necessary supporting structure in which malignant cells can proliferate and, on the

other hand, is the structure supplying malignant cells with nutrients, oxygen and water, and removing their waste products.

The primary signs of the stroma emerge in the isolated micro cavity, where the fibroblasts produce collagen fibres, which are a connecting component for malignant cells in the malignant “germ”. After the destruction of the micro cavity’s coat and the outgoing of malignant cells into the intercellular space, the active “construction” of a malignant neoplasm begins.

During this period malignant cells, using the surrounding connective tissue, are actively involved in the formation of stroma. They:
- stimulate the proliferation of connective tissue cells according to the paracrine regulatory mechanism and produce growth factors and oncoproteins;
- stimulate the synthesis and secretion of the extracellular matrix components by the connective tissue cells;
- secrete certain components of the extracellular matrix;
- produce enzymes (collagenases and others), their inhibitors and activators that help or inhibit the invasive growth of the malignant focus.

Later, the stroma of the primary focus grows and develops together with the tumour and thus is its own stroma. Stromal elements of a tumour are cells and the extracellular matrix of connective tissue, blood vessels and nerve-endings. The extracellular matrix of tumours is represented by two structural components: basement membranes and the interstitial connective tissue matrix. The coat of an isolated micro cavity transforms into the elements of the stroma.

Malignant tumours often form the stroma, which is dominated by the type of collagen of stroma of the related organ at the stage of its embryonic development: in stroma of lung cancer the collagen type III is predominant, in renal cell carcinoma and nephroblastoma - type IV, etc.

The growth of the tumour stroma occurs with the growth of its parenchyma. However, soon after reaching a certain volume of the malignant focus its surface area appears inadequate for the even diffusion of nutrients and waste products. Under these circumstances, the tumour periphery’s cells are located in favourable conditions and retain the ability to proliferate. At the

same time, the cells of the central parts are short of nutrients and oxygen, are subjected to dystrophic alterations and die as a result of necrosis. Proliferation activity of cells adjacent to the zone of necrosis is extremely low.

**Parenchyma**

The basis of the malignant focus's parenchyma is actually two types of malignant cells, the division of which can be made according to the key characteristics: "germination", activity of proliferation, survivability, capability for metastasis etc.

**1. The first type** - true malignant cells born as the result of proliferation of the "germinated" malignant stem cell in an isolated micro cavity. After the destruction of the micro cavity's coat, the first generation of malignant stem cells goes out into the intercellular space and reproduces. As a result of malignant progression due to genotypic instability and the influence of the microenvironment, the **second generation** of malignant stem cells emerges in the primary focus.

True malignant cells have hereditary genotypic alterations and phenotypic specifics, which can alter following changes in the microenvironment conditions. They have all the basic properties appropriate to malignant cells:
- they intensively reproduce (proliferate);
- they stimulate angioneogenesis;
- they invasively grow;
- they produce growth factors and release them into the intercellular space;
- they autonomously enter the bloodstream;
- they circulate for a long time through the host-body in the bloodstream;
- they organise metastasis;
- they influence haematopoiesis, homeostasis, immunity etc.

**2. The second type** – conditionally malignant cells, which arise in zones of growth of the restorative proliferation under the influence of growth factors of the true malignant cells. These are mainly phenotypically changed cells of the tectorial or glandular epithelium. Quantitatively, they grow due to proliferation of cambial poorly differentiated cells of intestinal crypts or the neck of glands, but only in their customary environment: the cavity of

an exocrine gland or on the basement membrane. If the integrity of the basement membrane is breached, they are able to go out into the intercellular space, but they do not have the abilities inherent in true malignant cells:
- they are not able to perform an active reproduction (proliferation);
- they do not produce active substances that stimulate angioneogenesis;
- they are not capable of invasive growth;
- passively “dropping” into the bloodstream cell by cell (or by conglomerate), they die immediately or during the circulation through the body in the bloodstream;
- they cannot organise a metastasis.

The conditionally malignant cells die by necrosis, thus stimulating the process of chronic inflammation and the growth of stroma, on account of the inflammatory mediators. After the death of conditionally malignant cells, a zone of necrosis in the centre of the primary focus emerges, performing the function of a framework. Large molecules emerging with the death of conditionally malignant cells are captured and phagocytosed by true malignant cells (cannibalism).

**Proportion of parenchyma and stroma**

Abnormality in the proportion of stroma and parenchyma, the nature of tumour vascularization and also the specifics of the intermediate substances’ development, are related to structural atypism of malignant neoplasmata.

It is known that the parenchyma and stroma in any organ or tissue, including those in a malignant tumour, are a single unit. In this case, the stroma is present in the primary focus initially. In other words, the stroma of a malignant tumour, as the essential structure providing nutrition, homeostasis and excretion of metabolic products, has initially surrounded an isolated micro cavity in which the “germination” of a primary malignant stem cell has occurred. Consistently, the same stroma also surrounded the malignant “germ” and after the exit of the **first generation** of malignant stem cells into the intercellular space, it actively and directly participated in the formation and growth of the primary malignant focus.

Hence the morphological character of the primary malignant focus may point, directly or indirectly, to the original location of an isolated micro cavity and to the beginning of the formation of the primary focus. Given that the structure of coats of the hollow organs have their own specifics, let us try to compare the morphological characteristics of a primary malignant focus and coats, taking the stomach as an example:

**1. Mucinous stomach carcinoma** is characterised by scarcity of stroma and a large number of parenchyma with malignant cells producing mucus in large quantities (Fig. 12).

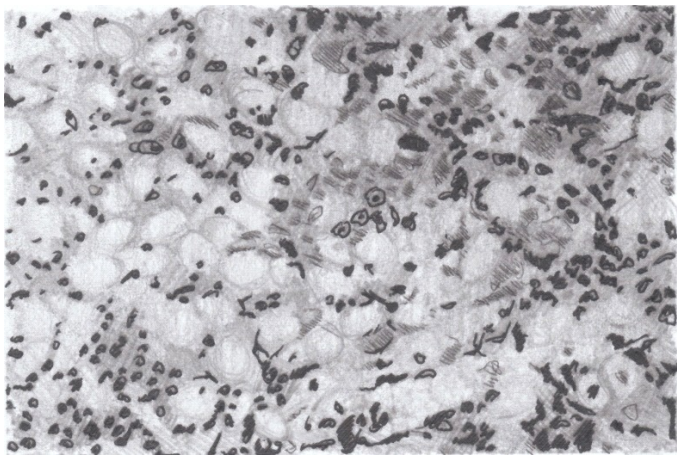


Fig. 12. Mucinous stomach carcinoma (N.N. Petrov, S.A. Kholdin, 1952, vol. 2, p. 685).

The fine loose connective tissue is under the basement membrane in the area of prismatic cells producing mucus and in the folds of its own layer of the mucous membrane. It is here that an isolated micro cavity has localised and it is this tissue which was used by the malignant process to form the stroma of the primary focus. Phenotypic similarity of malignant cells with glandular cells, producing mucus, is a result of mesenchymal-epithelial transition or appositional growth.

**2. Scirrhus of stomach** is dense, whitish and characterised by an abundance of elastic fibres and a small number of cellular elements (Fig. 13).

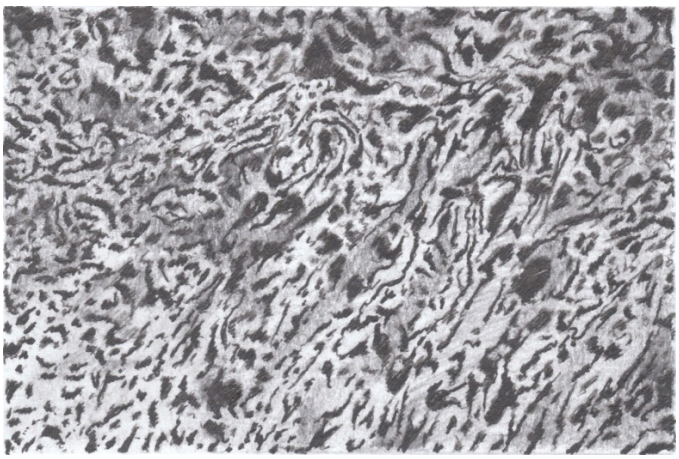


Fig. 13. Scirrhous cancer of the stomach (N.N. Petrov, S.A. Kholdin, 1952, vol. 2, p. 515).

In the structure of the gastric mucosa, the elastic fibres are in the submucous base (coat). So an isolated micro cavity and, consequently, the primary focus are localised in the submucosa base (coat). Severe degenerative alterations of the stroma, its hyalinosis and sclerosis preceded the development of this form of malignant neoplasm, contributed to the structural modification of the glandular parenchyma and caused scirrhus-like growth of the malignant focus.

**Angioneogenesis**

The growth of a malignant tumour depends on the degree of development of its vascular network. In a focus with a diameter of less than 1-2 mm the nutrients and oxygen inflow from the intercellular fluid by diffusion. For nutrition of larger neplasmata it requires vascularization of their tissue.

The blood vessels feeding the tumour were initially the existing vessels of the surrounding tissue, then newly formed blood vessels emerge, which differ significantly from the normal ones. In this case, the faster the tumour grows, the more imperfect is the structure of its vessels.

Angioneogenesis in a malignant focus is provided by a group of angiogenic growth factors. Their group consists of fibroblasts, endothelium, vessels of glioma, keratinocytes, epidermoid growth factor, angiogenine, some colony-stimulating bone marrow factors etc.

Newly formed vessels, sinusoids, enter into the postcapillaries or venules of the microcirculation bed (Fig. 14). As stated above, migration of the mononuclear cell from the vascular bed to the inflammation focus occurs also through tracts of the microcirculation bed, which have endothelium of the second type – these are postcapillaries or venules. Here is where the endothelium is most sensitive to stimuli, to which it responds by growth of adhesion and contractility; right in these locations a mononuclear cell exits from the vessel to the focus of inflammation, and the infiltration of malignant cells from the primary malignant focus into the bloodstream takes place.

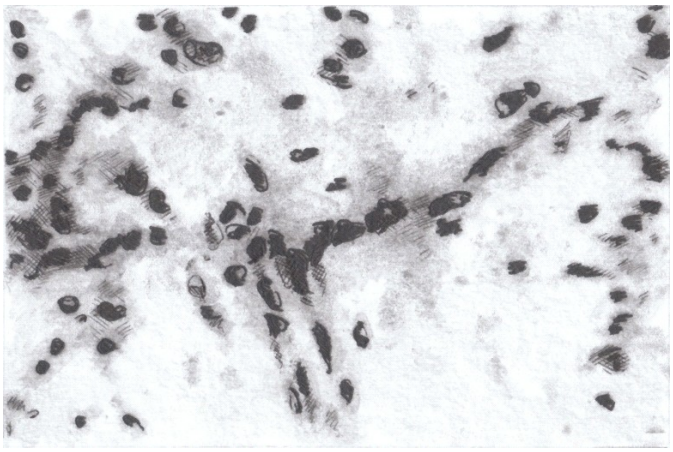


Fig. 14. Cancer of the larynx (I.A. Kraevskiy, A.V. Smolyaninova, D.S. Sarkisova, 1993, Vol. 1., p. 151): angioneogenesis (downward from the centre).

Many of the newly formed blood vessels are discerned by their considerable structural atypism. They are channels lined with endothelial cells of irregular shape and surrounded by developed perivascular connective tissue. Often their walls are poorly developed and malignant cells tightly surround the blood vessels; in some cases the endothelium may be partly absent, so the malignant cells themselves are involved in the lining of the capillaries; often there are secondary degenerative alterations (fibrinoid necrosis, hyaline impregnation).

**Immunomorphology**

***Prehn R.T. (1971):*** *many lymphocytes - inhibition of the tumour; few lymphocytes - stimulation of the tumour*.

The composition of wandering cells of the tumour’s stroma is very diverse and includes a different quantity of lymphocytes,

neutrophils, eosinophils, plasma cells, macrophages and giant cells of foreign bodies.

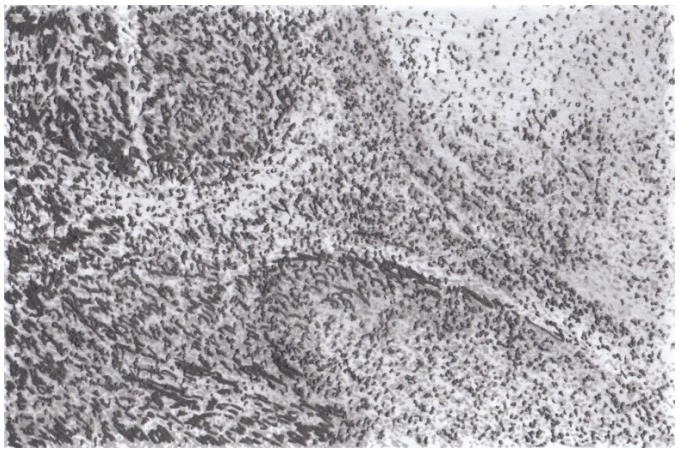


Fig. 15. Cancer of the larynx (I.A. Kraevskiy, A.V. Smolyaninova, D.S. Sarkisova, 1993, Vol. 1., p. 122): lymphoid-plasmocytic infiltration and a reactive lymph follicle in the growth zone.

Infiltration of a malignant focus by the host-body cells is a manifestation of immuneologic reactions to the presence of altered cells. The degree and the nature of the infiltration of a tumour by cellular elements of the organism's immune system may be an indicator of the aggressiveness of the neoplasm.

In slowly growing, highly differentiated malignant neoplasmata the lymphocytes are present in greater amounts than in the fast-growing ones (Fig. 15). With intensively infiltrating tumours the prognosis is usually better than with weak reaction of the organism or with its absence. The degree of infiltration of the tumour depends on its level of differentiation. There is an inverse relationship between the degree of infiltration of the tumour and its clinical aggressiveness. There is a correlation between the nature of the neoplasm's metastasis and the reaction of the host-body to it.

**Features**

1. Relative autonomy and unregulated growth.

2. Anaplasia or simplification of the structural and chemical organisation, reducing the differentiation of tumour tissue, which makes it closer to the embryonic one regarding a number of attributes and properties.

3. Heritability of alterations – in the process of reproduction a characteristic clone of malignant cells forms, which gives rise to the primary focus.

4. Invasive destructive growth – the main criterion of malignancy.

5. Embryolisation – emergence of embryonic antigens, embryonic forms of isoenzymes or embryonic variants of some proteins in malignant tumours.

## 2.2 PROTOTYPE

Making a single, universal scheme of structural organisation of the primary focus of various malignant diseases is a challenging objective due to the peculiar structure of its particular forms.

The primary malignant focus is an independent structural unit, which contains all the classic features of tissue, has an autonomous reproduction and the ability to spread out in the host-body.

The structural organisation and functioning of red bone marrow is the prototype of the structure and principle of operation of the primary malignant focus. Malignant cells attempt to build a malignant neoplasm according to a structural organisation which is known to them.

This assertion is based on the following factual comparisons:

1. Haematopoiesis in the red bone marrow takes place in islets, which are cells grouped according to the haematopoiesis shoots.

Malignant cells in malignant tumours are also located in aggregates or islets, in which they are grouped by their specific characteristics, and in the case of polyclonal development of the malignant focus, by the clones' shoots.

2. In red bone marrow the precursor-cells and developing cells are arranged as follows: in the centre – dividing and immature cells; in the periphery near the walls of the sinusoids – more mature cells, which penetrate into the bloodstream to perform their functions.

In a malignant tumour, a similar arrangement of cells is in agglomerations or aggregates: in the centre – dividing and immature cells; in the periphery near the walls of the newly formed blood vessels-sinusoids – more mature cells, which penetrate into the bloodstream to perform their functions.

3. The red bone marrow, as a highly vascularized organ, connects to the bloodstream through the capillary network. There are two types of capillaries: feeding (conventional) and functional (sinusoids); the latter flows into the common vessel: the central vein. The sinusoids' lay is radial, the haematopoietic tissue is between them.

A malignant neoplasm is poorly vascularized and uses the capillary network of the host-body for its nutrition. Given that these capillaries are not suitable for the penetration of malignant cells into the bloodstream, and major veins are absent, a mechanism of angioneogenesis emerges, as a result of which canals-sinusoids are formed, flowing into the venous part of the microcirculation – postcapillary and venule. Through these canals the malignant cells, one by one or in small groups, enter the bloodstream and circulate in the host-body. Of course, the newly formed blood vessels - canals-sinusoids - are far from perfect compared to the sinusoids of the red bone marrow, but are quite suitable for the task of penetration of malignant cells into the bloodstream.

4. The stromal microenvironment plays an important role in the regulation of proliferation and differentiation of haematopoietic cells in the red bone marrow.

There is parenchyma and stroma in malignant neoplasm, which are integral parts of the structural organisation of any tissue, while there is a close correlation and interdependent influence of parenchyma and stroma.

There are identical components in the red bone marrow and in malignant neoplasm, which are as follows:
- cellular components: fibroblasts, fat cells, macrophages, osteoblasts and endothelial cells;
- the extracellular matrix, which consists of the products of stromal cells' secretion: collagen, fibronectin, laminin, glycosaminoglycans and other protein components.

5. According to statistics, in the early stages of a malignant focus formation, malignant cells are already observable in the lymph nodes and in the bone marrow. This process can be called a “returning home – homing”. Indeed, in normal circumstances promonocytes and monocytes come out from a blood vessel into the tissues, can no longer return back to the bloodstream and the bone marrow, but “want” to do this. Therefore, a “germinated” malignant stem cell by proliferation, invasive growth and angioneogenesis makes for itself a mechanism of “returning home – homing”.

Homing (returning home) - the ability of cells to migrate or “return back” to the tissue from which they were received and the integration of cells in these tissues through specific receptors of the homing. The homing process involves three stages: the migration of cells along the bloodstream, their transmigration into tissues through the capillary walls, and the retention of cells in the tissue through specific receptors.

6. All blood cells originated from the pluripotent haematopoietic stem cell. Subsequently, normal haematopoiesis is polyclonal, i.e. it is carried out simultaneously by many clones.

In 80% of cases the malignant neoplasmata are monoclonal, i.e. all malignant cells are descended from a single primary malignant stem cell. In 20% of cases the malignant process is polyclonal, i.e. it is carried out simultaneously by two or more clones.

7. During embryonic haematopoiesis a special substance is produced – “angiogenesis factor” for the development of blood vessels.

Malignant cells also produce a special substance – “angiogenesis factor” by making favourable conditions for the increased inflow of nutrients and for the “washout” of malignant cells into the bloodstream.

8. The quantity of haematopoietic cells is approximately the same throughout the life of the organism.

The total quantity of malignant cells in a malignant neoplasm, upon reaching a certain level, is also maintained approximately the same, because the number of dead cells in the centre of the

tumour is offset by a proliferation in the periphery, where the feeding vessels are located.

9. In the red bone marrow, as a result of proliferation and with the help of sinusoids, the mature cells enter the bloodstream in quite large volumes.

In a malignant neoplasm, also as a result of proliferation, invasive growth, appositional growth and angioneogenesis, the malignant cells enter the bloodstream in quite large volumes.

10. The red bone marrow through the blood cells and the malignant focus through the malignant cells definitely affect the vital processes in the host-body: haematopoiesis, homeostasis, immunity etc.

11. The factors regulating the work (growth, differentiation) and the functioning of the red bone marrow and the malignant focus are almost identical.

**Thus,** organisation of the primary malignant focus is an important stage in the development of the malignant process.

# 3. GROWTH FORMS

During the development of the malignant process, the formation and growth of the primary focus occurs. The manifestations of the growth of the primary focus depend on many factors, the main ones being as follows:

1. “Reliable” isolation of a malignant “germ”.
2. Proteolytic capabilities of malignant cells.
3. Remote location of the primary focus in relation to the basement membrane.
4. The structural features of the connective tissue.

## 3.1 EXPANSIVE GROWTH

Expansive growth is increase in the size of a malignant “germ” due to active proliferation of malignant cells, without destroying the integrity of the insulating coat (Fig. 16).

In this case, the primary focus is surrounded by the same coat that has been around the micro cavity and is growing “out of itself”, pushing away the surrounding tissue. As a result of atrophy of the parenchymal cells and stroma surrounding the tumour, as well as the ability of the host-body to limit the malignant process, it increases the strength of the coat which surrounds it.

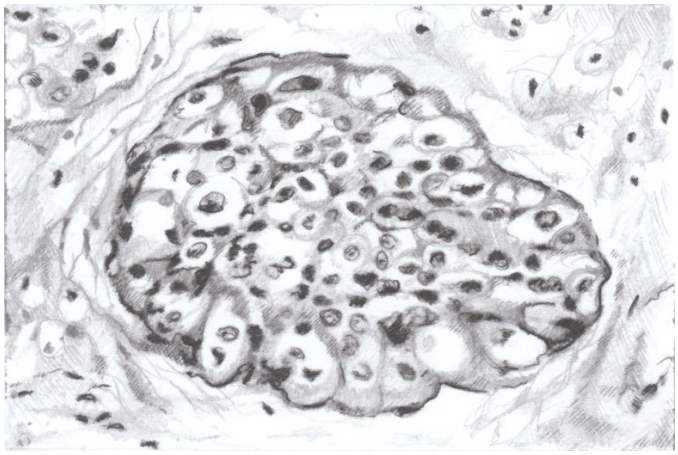


Fig. 16. Adenocarcinoma. Expansive growth.

Expansive growth is slow, and is a feature of some malignant solid tumours: kidney cancer, thyroid cancer etc. With expansive growth there is no absolute isolation of the primary focus, because its development requires the supply of food, water, oxygen and the removing of waste products.

With expansive growth, there are no blood vessels and no nerve endings within the micro cavity or at least they are in their infancy, but there are the rudiments of connective tissue produced by fibroblasts and the amorphous substance, which are the binding material for malignant cells.

Increasing the number of malignant cells inside the micro cavity can lead to the following variants of the further development of the malignant “germ”:

1. Increasing the size of an isolated micro cavity to the greatest possible extent.

2. Breaching the integrity of the insulating coat and breaching the permeability of the basement membrane, followed by the malignant cells’ exit into the basement membrane and formation

of the “carcinoma in situ”. Here the basement membrane may participate in formation of the insulating coat.

3. The destruction of the insulating coat followed by the malignant cells’ exit into the intercellular space and formation of the primary malignant focus.

## 3.2 APPOSITIONAL GROWTH

***Waldeyer H.W. (1867):*** *on the border with the malignant neoplasm, the glandular tubes are partially lined by cells which are stained with carmine stronger than normal ones. These alterations are considered as typical initial manifestations of malignancy*.

***Bohmig R. (1950):*** *malignancy of the glandular epithelium is not random, but arises multicentrically it begins always on the same side in the top corner of two adjacent tubes, not passing over to the neighbouring one*.

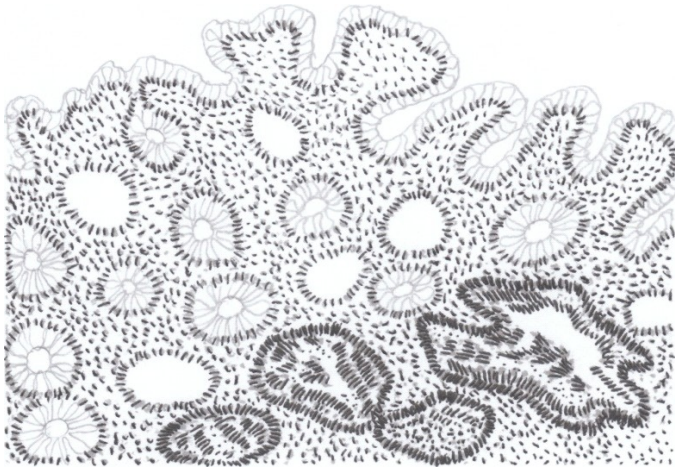
Appositional growth is a form of growth of the primary focus by malignant transformation of normal proliferating coating and/or glandular epithelium under the influence of growth factors of true malignant cells (Fig. 17). In fact, this is a mass formation of conditionally malignant cells from normal epithelial cells and to a greater extent it takes place in the system of exocrine glands.

With the increase of critical mass, true malignant cells produce and release growth factors into the intercellular space. The latter, affecting the cambial zone of growth in the bottom of the crypts or the neck of exocrine glands, provide stimuli involving the cell in a cycle of fission; because stimulating the division of a normal proliferating somatic cell requires availability of growth factors. In this case, the signal for the cell division always comes “from outside”, so the normal cell cannot be autonomous.

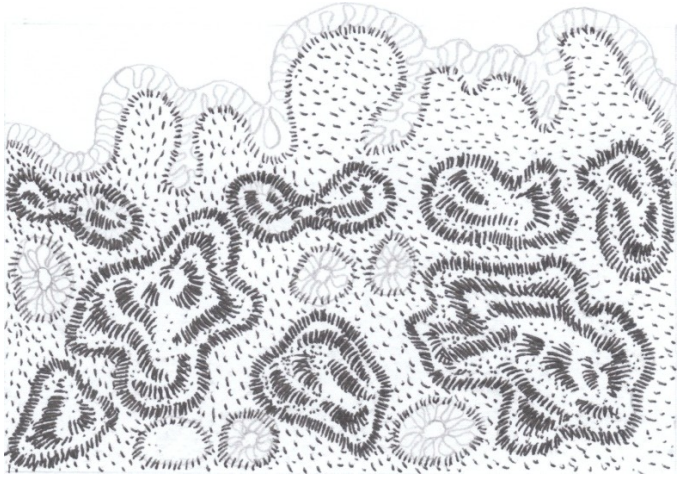
The depth of an isolated “micro cavity” depends on the shape of the mucous membranes and also, respectively, the depth of a malignant focus in relation to the basement membrane, and consequently, the degree of influence of the true malignant cells’ growth factors on the epithelial cells.

If the shape of the mucous membrane is “flat” (the ducts of mammary glands and prostate, cervix uteri etc.), the micro cavity and, respectively, the primary focus may be formed under the basement membrane, perhaps even with participation of the basement membrane in the formation of the coat of an isolated micro cavity. In this case, it is a manifestation of the appositional growth of tectorial epithelium.

If the shape of the mucous membrane is “complex” (stomach, colon, lung etc.), then the isolated micro cavity and, respectively, the primary focus are formed or lie deep in the connective tissue, somewhere on the level of exocrine glands. In this case, a manifestation of appositional growth of glandular epithelium has to be expected.



A



B

Fig. 17 (A, B). Adenocarcinoma. Appositional growth.

Under the influence of growth factors, the proliferating exocrine epithelium alters phenotypically; its reproduction activates. As a result, cylindrical simple or cubical simple epithelium of a glandular tube transforms into a multirowed atypical or malignant epithelium.

Generation of atypical epithelium forms a sharp border with the normal secretory epithelium, which constitutes the end parts of glands. At the junction the possibility of transition from one generation to another is completely excluded.
The process of tissue development follows the way of gradual displacement of the normal epithelium by atypical epithelium, which demonstrates busy life activities, a reflection of increased intracellular metabolism. However, despite live metabolism and intensive processes the atypical epithelium has sharply reduced capability for regeneration.

Subsequently, malignant cells grow in the glands, spread beyond the cervix and seize its body and the entire gland. The normal picture of glands is sharply disrupted, their numbers are often reduced, and those remaining are transformed into large crypts, cysts or become a tube, formed and filled with homogeneous malignant cells.

Due to the involvement of many glands in the process of malignant transformation, an increase occurs in the tissue mass of tumours by appositional effect. Malignantly transformed glandular cells overflow the cavity of a gland, destroy the basement membrane, go out into the intercellular space and join the growing structure of the primary malignant focus. After exit beyond the destroyed exocrine gland, conditionally malignant cells may also stay in agglomerations as having the rights of “well-connected” ones.

Conditionally malignant cells are the main contenders for mass death in the primary focus. As a result of their death, there occurs stimulation of inflammatory mediators and the growth of stroma of the malignant focus – formation of the necrotic frame. Large molecules of dead cells capture the true malignant cells and use them as a source of energy and building materials (cannibalism). Once entered into the blood vessels, conditionally

malignant cells die at different stages of circulation, being unable to organise metastasis.

With the transformation of normal epithelium into the atypical one in the process of development, the secretory activity has been lost. Therefore, being on the mucosal surface in direct contact with external factors, the atypical epithelium shows little resistance, is easily rejected and often not formed again. This leads to the fact that the malignant areas of mucosa are often deprived of the epithelial lining and on the wound surface a significant amount of detritus accumulates, produced by the dead elements.

## 3.3 INVASIVE GROWTH

Invasion – contagion of animal parasites.

Invasive or infiltrating growth is characterised by the fact that the true malignant cells of the primary focus grow in the surrounding tissues beyond its borders and destroy them. Invasive destructive growth is the main criterion of malignancy.

The invasion typically takes a soft option of going via the inter-tissue holes, along the nerve fibres, blood and lymphatic vessels (Fig. 18, 19). Masses of true malignant cells destroy them, grow into the loose connective tissue and penetrate into the blood stream and lymph.

Invasive growth is an active penetration of true malignant cells through the tissue barriers. The malignant focus grows into surrounding tissues not because it presses on them, but it acquires this ability as the result of additional genetic and biochemical alterations. If a capsule of an organ, a membrane or other solid tissues cross the path of the invasion, the malignant cells initially spread over their surface, and then growing through the capsule or membrane, penetrate deep into the organ.

Invasive growth is a result of changes in the sensitivity of true malignant cells to activating and inhibitory signals, and in the imbalance between the processes regulated by them. It is clear that with such a form of growth the boundaries of a malignant focus are vague and misty.

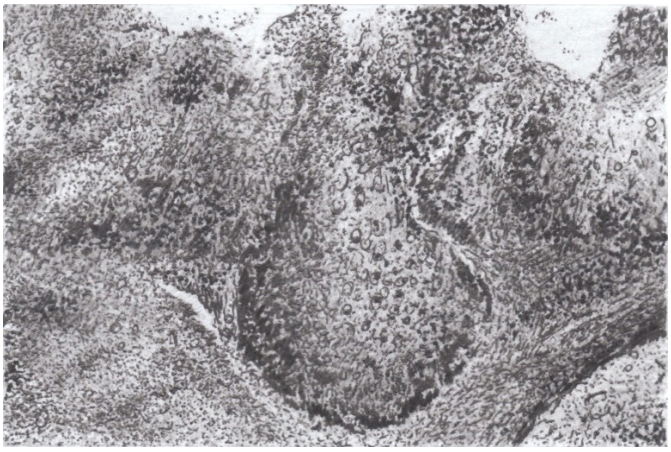


Fig. 18. Micro-carcinoma (I.A. Kraevskiy, A.V. Smolyaninova, D.S. Sarkisova, 1993, Vol. 1., p. 229): the invasion of malignant cells occurs through the destroyed isolating coat of a micro cavity (from the top down) towards the mucous membrane.

The invasion of true malignant cells is provided by the following factors:

- the growth of their critical mass;
- weakening contacts between them;
- increased mobility;
- change in pH level (acid – more often, alkaline – seldom) in the area of a malignant focus and, as a consequence, “escape” of malignant cells from this area;
- increased abilities to bind to components of extracellular matrix (collagen, laminin, fibronectin);
- increased secretion of proteolytic enzymes and their activators;
- they use products of the destruction of cellular and non-cellular structures as a source of energy and building material.

Fast invasive growth of a malignant focus is specific for immature malignant tumours. Depending on the number of tumour foci one speaks of unicentric (one focus) or multicentric (multiple foci) growth.

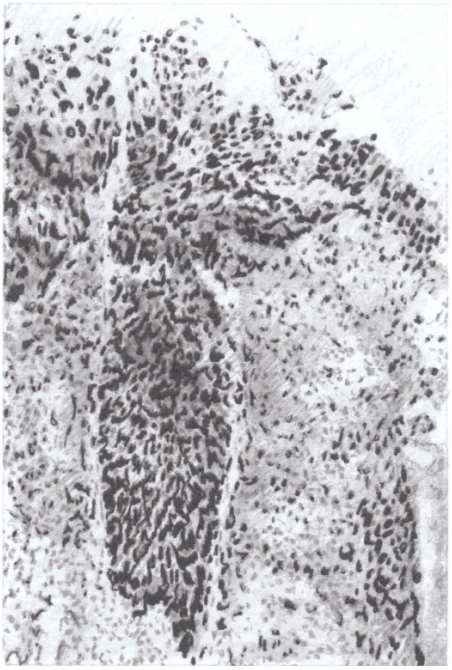


Fig. 19. Micro-carcinoma (I.A. Kraevskiy, A.V. Smolyaninova, D.S. Sarkisova, 1993, Vol. 1., p.228): the drop-like invasion of malignant cells occurs through the destroyed insulating coat of a micro cavity (from the bottom up) towards the serous membrane.

**Thus,** the expansive growth of a malignant focus is similar to the growth of benign tumours. The volume of a primary malignant focus is increased by proliferation and appositional growth, while the penetration into surrounding tissues results from the invasive growth of malignant cells. In relation to the lumen of a hollow organ the growth of the primary tumour may be endophytic or exophytic:

1. Endophytic growth - invasive growth of the tumour into the wall of an organ.
2. Exophytic growth - expansive growth of the tumour into the cavity of an organ.

# 4. FEATURES OF GROWTH

## 4.1 AUTONOMY

The autonomous growth of a malignant neoplasm is characterised by a lack of control of proliferation, differentiation and maturation of cells by the host-body. This does not mean that the malignant cells are in some proliferation chaos. In fact, tumour cells pass to use autocrine or paracrine mechanisms to regulate their growth.

In the case of autocrine stimulation of growth, the malignant cell itself produces growth factors or oncoprotein-analogues of growth factors, and also receptors or oncoprotein-analogues of growth factor receptors. This happens, for example, in the case of small cell lung cancer, whose cells produce growth hormone (bombesin) and at the same time receptors to it. In this process a paracrine stimulation also occurs, as bombesin may also interact with neighbouring cells. An example of the paracrine stimulation of tumour is the production of insulin-like growth factor 2 by fibroblasts of the stroma of lung cancer. The growth factor interacts with receptors of malignant cells and stimulates their proliferation.

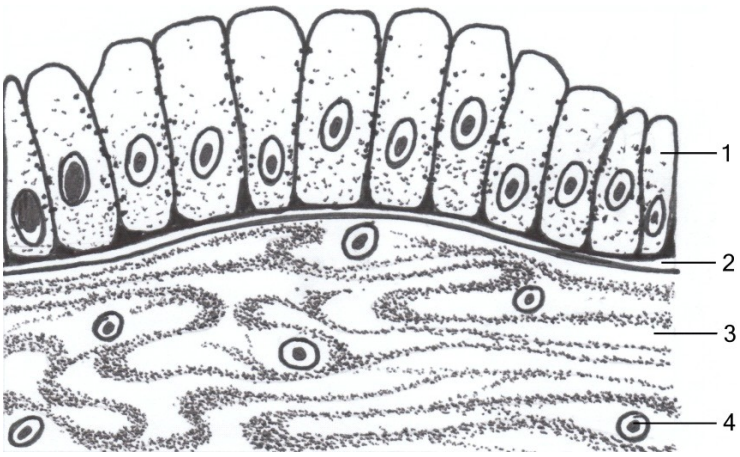
The autonomous growth of tumours is expressed in the loss of contact inhibition and immortality of malignant cells, that can be attributed to the transition of cells to autocrine and paracrine forms of their growth's regulation. The autonomy of the tumour is relative, since the tumour tissue constantly receives from the organism various nutrients, oxygen, hormones and cytokines, which are brought by the blood stream. In addition, it undergoes the impact of the immune system and adjacent non-tumour surrounding tissue.

**Thus,** the autonomy of the malignant focus should not be understood as the complete independence of malignant cells from the host-body, but as the malignant cells' acquisition of the ability for self-governance.

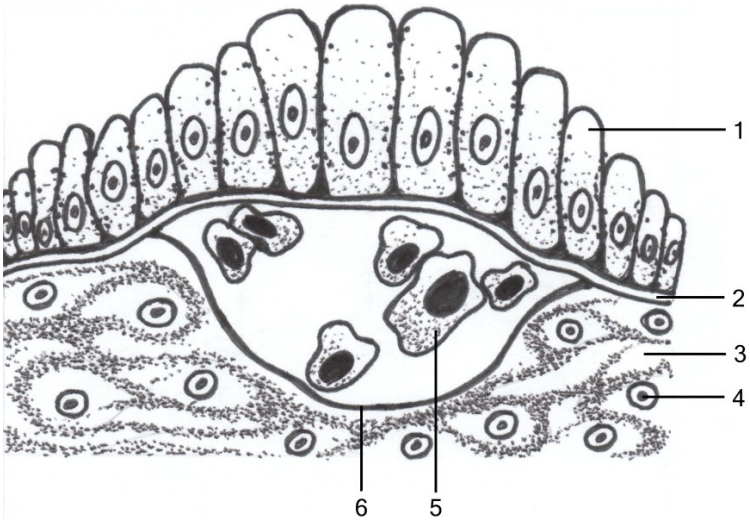
## 4.2 " CARCINOMA IN SITU" (CIS)

***Cheatle G.L., Cutler M. (1931):*** *"carcinoma in situ" is a form of cancer, initially represented by a pool of malignant cells, bound within epithelium; they do not involve the basement membrane in the process, but potentially they are capable of invasion*. This assumption prevails to this day, although many questions arising in this case remain unanswered.

As mentioned above, in relation to the basement membrane the isolated micro cavity’s depth of lie depends mainly on the shape of the mucous membrane. In the ducts of mammary glands, prostate, cervix uteri, etc. the shape of the mucous membrane is “flat”, so the micro cavity can be formed under the basement membrane. In this way, the basement membrane may participate in the formation of a micro cavity’s coat (Fig. 20).



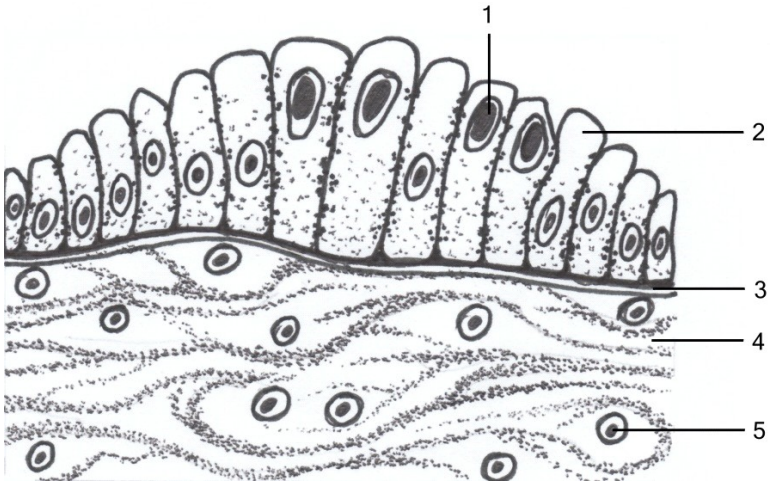

A




B

Fig. 20 (A, B). Scheme of formation of the "Carcinoma in situ":
A – fragment of normal mucosa: 1 - epithelium, 2 - basement membrane, 3 - connective tissue, 4 - lymphocyte.

B – isolated micro cavity under the basement membrane: 1 - enlarged epithelium, 2 - basement membrane, 3 - connective tissue, 4 - lymphocyte, 5 - malignant cell, 6 – coat of the micro cavity.




C

Fig. 20 (C). Scheme of formation of CIS:
C – “Emigration” of malignant cells onto the basement membrane with the formation of “carcinoma in situ”: 1 - true malignant cell, 2 - malignant cell, 3 - basement membrane, 4 - connective tissue, 5 - lymphocyte (lymphoid infiltrate).

In this case, increased size of the micro cavity is not prevalent, since with the increasing mass of malignant cells their “emigration” to the basement membrane and formation of the “carcinoma in situ“ (CIS) occur. At the same time, the growth factors secreted by the true malignant cells affect cambial elements of the epithelial cells’ growth area, thus transforming them into the atypical epithelium. Atypical epithelium consists of relatively malignant cells having only phenotypic alterations and not capable of proliferation.

As a result, there are two types of cells on the basement membrane: the true and the conditionally malignant cells, differing from each other in key attributes. The main part is

conditionally malignant cells, the smaller part - migrated malignant cells. This combination creates an imbalance in the cytological and functional features:

- conditionally malignant cells do not proliferate – the nucleus is located in the centre or in the basement part and organelles are in the apical part; they are poorly resistant to aggressive impacts from the environment;

- true malignant cells proliferate, but very rarely – the nucleus is located in the centre, in the basement or in the apical part, and organelles, respectively, are in the apical or basement part; they are poorly resistant to aggressive impacts from the environment.

Let us consider the specifics of the emergence and development of CIS in various organs and tissues of different ages and gender groups:

1. It is known that the frequency of detection of CIS in the prostate increases with age, reaching its peak in the sixth decade.

**Question:** why CIS is more common in the prostate gland of patients beyond 50 years of age than in younger patients?

**Answer:** due to specifics of the morphological structure of the prostate and the capabilities of the host-body.

Here the tectorial epithelium is on a layer of basement cells. While ageing, due to dilution and partial disappearance of basement cells, as well as increased vascularization around such foci, the tumour is growing faster as the obstacle of the basement membrane is weaker, but the ability for delimitation and “emigration” of the malignant “germ” is quite good. Specifics of the morphological structure of the prostate, as well as the progress of the malignant process and the capabilities of the host-body, contribute to early and effective delimitation of the malignant focus.

2. It is known that at the present time CIS is detected in 20-40% of cases of all newly diagnosed breast cancers, predominantly in younger women.

**Question:** why is breast CIS more common in younger women?

**Answer:** due to specifics of the morphological structure of the mammary gland and the capabilities of the host-body.

Younger age in women predisposes an increased ability to delimitation and “emigration” of malignant “germ”. Thus, morphological features of the mammary gland, the progress of the malignant process and the capabilities of the host-body contribute to an inclination for earlier and effective delimitation of the malignant focus.

3. It is known that the normal tectorial epithelium is located on the basement membrane, closely adjoining each other. The epithelium has a prismatic shape, its nucleus is in the centre or in the basement part respectively, and organelles are in the apical part.

Malignant cells located on the basement membrane and referred to as the primary cancer epithelium, are a layer having a considerable thickness and formed by a single row of high and relatively narrow cells. All cells’ nuclei are elongated and located in the basement, middle or apical parts. The organelles are located in the apical or basement part of the cell. Such an alteration for the malignant cells that lie on the surface of the mucosa, is determinate. Location of the nucleus in the apical and organelles in the basement part, is explained by the inversion of malignant cells. Mitotic figures are extremely rare.

**Question:** why in the case of CIS part of the malignant cells have a nucleus located in the apical part and organelles located in the basement part – an “inverted” position of the nucleus and organelles?

**Answer:** initially the true malignant cells are under the basement membrane and their nuclei are located in the basement parts and the organelles are in the apical parts of cells in relation to the basement membrane. Therefore, the true malignant cells under the basement membrane and cells of tectorial epithelium on the basement membrane are in a “mirror” location to each other.

In the case of “emigration” of the true malignant cells onto the basement membrane, some of them retain the location of the nucleus and organelles, as when they were under the basement

membrane. But now, lying on the basement membrane, their nuclei are in the apical part and organelles in the basement part. That is how the “inverted” or “mirror” location of the nucleus and organelles is formed in some of the true malignant cells in relation to the cells of tectorial epithelium and other malignant cells.

4. It is known that the there are no intermediate cells on the basement membrane between malignant cells and the normal epithelium.

**Question:** why in the case of CIS are there no transitional forms between normal cells of the epithelium and malignant cells?

**Answer:** usually there is no continuous series of transitional forms between two different types of cells. The exceptions are those cases where the sequential alterations in the development of a one-cell form are observed.

5. It is known that in approximately 18% of cases of breast CIS the malignant cells are found under the basement membrane and in up to 30% of cases, in the peripheral blood.

**Question:** how are the malignant cells in the case of breast CIS detected under the basement membrane and in the peripheral blood?

**Answer:** the primary malignant focus was initially under the basement membrane, but during its “emigration” onto the basement membrane there was a loss of some malignant cells, followed by their partial “leaking” into the bloodstream.

6. It is known that the atypical epithelium is an unstable short-term structure. It is easily rejected and often not formed again, so it leads to loss of the mucosa of the epithelium lining.

**Question:** how, in difficult conditions of the environment with low regenerative capacity, can the atypical epithelium form CIS?

**Answer:** the “germination” of a malignant stem cell and proliferation of true malignant cells occur in optimal conditions – under the basement membrane in an isolated micro cavity. Then a formed clone of malignant cells stimulates the appositional

growth of epithelial cells and partially “emigrates” to the basement membrane.

**Thus**, the “carcinoma in situ” is an exophytic form of cancer in miniature.

## RÉSUMÉ

“Germinated” in isolation, a malignant stem cell initiates the growth of a malignant focus, which cannot progress successfully in isolation.

# CHAPTER V

# THE DEVELOPMENT OF THE MALIGNANT PROCESS

Initially, the malignant process is a local manifestation of a general disease of the organism. Subsequently, its unregulated development occurs in the form of expansive, appositional and invasive growth of the malignant focus, it spreads all over the host-organism and metastasis takes place; in this way the malignant process became dominant. Thereby, all its manifestations may be considered complications of the major general disease.

## 1. THE MALIGNANT PROCESS IS A SELF-CONTAINED SYSTEM

The malignant process, as a self-contained system, is characterised by its own control of proliferation, differentiation and maturation of malignant cells, their spread and metastasis,

as well as subduing the vital organs and systems of the host-organism.

## 1.1 A PROTOTYPE OF THE RELATIONSHIPS

The hormonal system may be used as a prototype of the relationships between malignant cells and non-cellular structures, as integral elements of the malignant process, as well as of the relationship with the host-body. A special feature of the hormonal system is that it reacts more slowly, but much longer, since as a result of evolution it has become more sophisticated in relations to the nervous system.

Compensating for hormone deficiency, the structural elements of the malignant process resume and/or strengthen their hormonal potential. By creating an endocrine profile in the host-body, the malignant process uses it as a tool to subdue and in future as a mechanism to regulate the vital organs and systems.

At the same time, the malignant process successfully competes with the normal hormonal, nervous and immunocompetent systems. The response of the malignant process to hormonal factors of the host-organism regulating proliferation, differentiation, maturation of cells and others, manifests itself either as a "deafness" or as a "hypersensitiveness". Such reaction is an integral part of the development of the malignant process as a self-contained system.

Table 1 shows a list (which is nowhere near a completed one) of tumours producing ectopic hormones. Ectopic hormones are those produced by tissue that normally does not secrete them, regardless of whether the ectopic focus is a glandular tissue or not.

Table 1

THE MAIN TUMOURS PRODUCING ECTOPIC HORMONES

| | Hormone | Tumours |
|---|---|---|
| 1 | ACTH (MSH) | Lung carcinoma, thymoma, islet cell tumours, pancreatic tumours, medullary |

| | | |
|---|---|---|
| | | thyroid carcinoma and rarely, other derivatives of neural crest. |
| 2 | Gonadotrophin | Tumours of trophoblastic origin, lung carcinoma, teratoma and other rare tumours such as hepatoblastoma in children. |
| 3 | Parathyroid hormone | Carcinoma of the lung, kidney and predominantly, ovary carcinoma. |
| 4 | Vasopressin | Lung carcinoma is the most common. |
| 5 | Hypoglycemia | Mesenchymal tumours, hepatoma, adrenal tumours and rarely, other tumours. |
| 6 | (?) Antivitamin D, hypophosphataemia | Mesenchymal tumours, carcinomas, haemangioma and various other tumours. |
| 7 | Serotonin | Lung carcinoma, thymoma, islet cells of pancreas, medullary thyroid carcinoma. |

Malignant tumours can synthesize embryonic proteins and embryonic isoenzymes. Embryonic protein – alpha-fetoprotein (AFP), is a specific component of embryonic serum and mammals' fetal serum. Its synthesis begins in the endoderm of an **embryo's yolk sac**, is completely terminated in the adult organism and only temporarily resumes in the case of some liver diseases: hepatocellular carcinoma and germinal teratoid carcinomas.

## 1.2 SELF-ORGANISATION AND SELF-REGULATION

The malignant process, as a complex dynamic system, is a self-organising system. Self-organised systems are those which, in the case of changing external and/or internal conditions of their functioning and development, are capable of maintaining or improving their organisation, taking into account their past experience, the signals of such changes being received through the feedback channels. Depending on the selection of a key group of properties, they are also called self-regulating or self-adjusting systems.

The problem of self-organisation and self-regulation is essential for understanding the evolution of emergence, growth and development of the malignant process. In this case, we are

talking about the processes of complication, resulting in the formation of highly ordered structures, qualitatively different from the original ones.

Gradually, under the influence of natural selection, the existing generation of malignant stem cells improves and new ones emerge. Of course, the new generations of malignant stem cells are improved self-organising systems of organic nature.

There are three main characteristics of self-organisation and self-regulation of the malignant process: homeostasis, feedback and information.

**1. Homeostasis** – is the relatively dynamic constancy of the composition and properties of the internal environment and sustainability of basic physiological functions. Homeostasis is the craving of a living system for maintaining the stability of its organisation. Homeostasis is inherent in any live substance and any living system. The pursuit of homeostasis is a very powerful factor of evolution, directly affecting the intensity of natural selection.

**2. Feedback** is the mechanism of a system's reaction to external stimuli. More generally we can say that feedback is a mechanism that determines the change of state, it is a reaction to external stimuli and it is determined by this reaction.

Metabolism is peculiar to the malignant process – the exchange of energy and substance is indispensable to its existence. One of the leading trends in the development of the malignant process is the pursuit of mostly using energy from the environment, which means the energy of the host-body. Moreover, the malignant process is eager to maintain homeostasis and to change its own system and environment systems in such a way as to direct the evolutionary process towards increasing its own ability to consume the energy and substance of the host-body.

**3. Information** is a reflected structure, reproducing the structure of the original one. Information is necessary to enable the actor to succeed in some of its purposeful activities. In particular, to be sustainable each emerging actor should have a stockpile of information to ensure reactions with a retaining function. The “germinated” primary malignant stem cell initiates

its growth and development as a self-contained system, so all this also applies to the malignant process.

The quality of information depends on the actor (malignant cells, malignant focus) and its ability to perceive and process information. The quality is assessed primarily by how knowledge obtained about the subject or the environment helps in the decision-making. The value and meaning of information is fully disclosed only when there is an objective.

The “germinated” malignant cell initially completely depends on the complexity of the environment and it adapts to these conditions. Its future evolution goes towards adapting the environment for its own development, as it is not burdened with functional responsibilities to the host-body. After accumulating a critical mass of malignant cells, there occurs a complete subordination of the vital organs and systems of the host-organism to them and reduction of their dependence on its protective forces.

There are two periods in the formation of a malignant process as a self-organising and self-regulating system:

**1. Short period** – a period of “germination” of a primary malignant stem cell, when transitional processes take place, accompanied by deviated development of a normal somatic proliferating cell from the typical state to the atypical (cancerous) one.

**2. Long period**- after the “germination” of a primary malignant stem cell and till the development of the final sustainable state of the entire developing system of a malignant process.

Further evolution of the malignant process goes towards the selection of those malignant cells which allow the quick and comprehensive assimilation of vital substances, putting the mechanisms of anaerobic and aerobic decomposition of these substances into the basis of the biochemical processes to receive the necessary life energy from the organic substances.

**Thus,** the malignant process is a self-contained system, which uses its own endocrine profile for self-organisation and self-regulation and also for relations with the host-body.

# 2. FEATURES OF THE MALIGNANT PROCESS

## 2.1 METASTASIS

Metastasis is organisation of the secondary focus (metastasis) in various tissues or target-organs of the host-body which are remote from the primary focus of the malignant process. Organisation of metastases characterises the development of a malignant process as a self-contained system, while the process goes on according to a certain programme and has its own specific morphological, biochemical, immunological and other signs.

Metastasis is affiliated with the spread of malignant cells from the primary tumour’s focus to other organs or tissues by the lymphatic and blood vessels, by perineural and implantation ways. The capability for metastasis is the distinguishing feature of the malignant process and the main method of dissemination of malignant cells separated from the primary focus (Fig. 21).

The indispensable conditions for metastasis are the invasive growth of the primary focus and angioneogenesis. Due to that, clear boundaries with the adjacent non-tumour tissue are absent because of the growth of malignant cells into it, while the developed vascular network and stroma emerge in the primary focus.

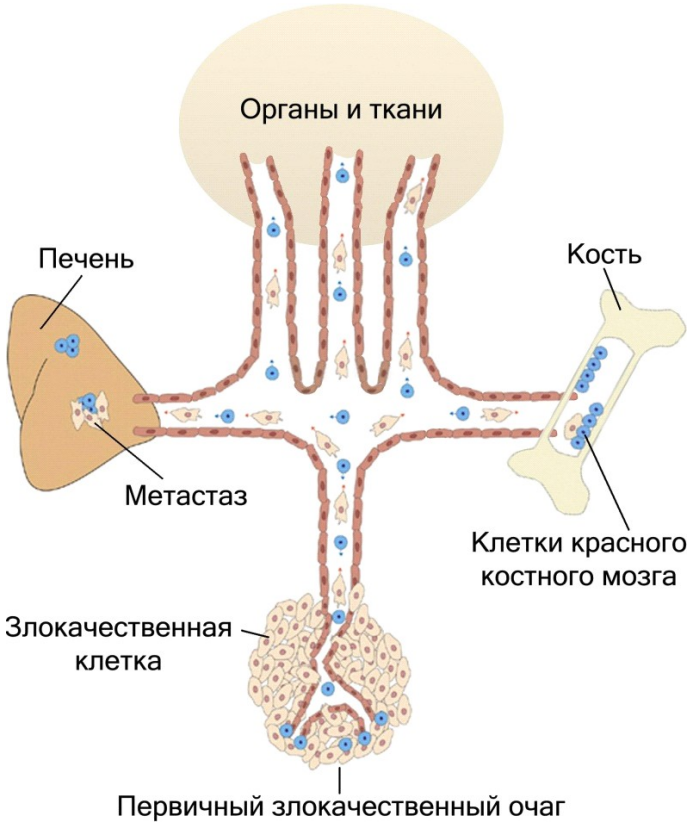



Fig. 21. Scheme of the metastasis

Metastasis starts very early, already underway in the process of the primary focus formation. Malignant cells are detected in the blood; in the case of a mass of malignant focus from 1mg to 1g, there are $10^6$ - $10^9$ malignant cells in it.

**Programme**

The programme of metastasis consists of many determinate, evolutionally conditioned and successive stages separated in time and space.

Stages of metastasis:

**First stage** – true malignant cells go out from the primary focus into the vascular bed, circulate through the host-organism and search for optimal tissue or target-organs for organisation of mature metastasis.

**Second stage** – exit of the red bone marrow haematopoietic stem cells into the bloodstream, then into the tissues or target-organs followed by the formation of a “pre-metastasis” bed.

**Third stage** – migration of a malignant stem cell from the vascular bed to the “pre-metastasis” bed, and organisation of mature metastasis.

**Prototype**

The prototype of metastasis is an embryonic haematopoiesis, whereby a consecutive change of stem cell generations occurs in three steps, with change of their localisation in organs and tissues involved in haematopoiesis:

**1st step:** the wall of yolk sac;

**2nd step:** liver, thymus, spleen and lymph nodes;

**3rd step:** red bone marrow.

Proof of this is the following:
- Firstly, metastases are organised in those tissues and target-organs that have been involved in the embryonic haematopoiesis: lymph nodes, bone marrow, liver and spleen. With this, the second generation of cancer stem cells located in the primary focus, like the generation of blood stem cells of the haematopoiesis, looks like “returning” into those same organs and tissues. Conditionally repeating the embryonic haematopoiesis, the true malignant cells organise mature metastasis, as a new focus of blood formation;
- Secondly, in those parts of tissues or target-organs where previously embryonic haematopoiesis was functioning, the **structural features remain** that contribute to formation of the “pre-metastasis” bed;
- Thirdly, most often, metastases are organised in those tissues and target-organs where a double type of blood circulation takes place: the lymph nodes, bone marrow, liver, spleen and lungs.

**Basis**

The basis of metastasis is the following:
- invasive growth of the primary malignant focus and angioneogenesis;
- ability of true malignant cells to penetrate into the bloodstream, to search for optimal tissues or target-organs with the aim to organise mature metastasis, to mark them remotely for the

subsequent arrival of haematopoietic stem cells of the bone marrow;
- ability of the malignant focus (malignant cells) to produce specific tumour-associated signals that launch the processes of migration of the bone marrow's haematopoietic stem cells, their adhesion and the formation of the "pre-metastasis" bed in a tissue or a target-organ;
- autonomous regulation of growth, development and dissemination of true malignant cells in the host-body: penetration into the vascular bed, circulation by the bloodstream, penetration into the tissues and target-organs, migration into the formed "pre-metastasis" bed and organisation of mature metastasis;
- malignant progression – a succession of generations of malignant stem cells;
- growing superiority of cellular and non-cellular structures of the malignant process over normal cellular and non-cellular structures of the vital organs and systems of the host-organism;
- formation of the malignant process's ability to monitor the basic vital organs and systems of the host-body and subsequently to subdue them.

**Mechanism**

Actually the mechanism of metastasis occurs in three stages, separated in time and space:

**First stage** - true malignant cells of the primary focus mark the tissue or the target-organ as a place of future metastasis.

Due to invasive growth and angioneogenesis, two groups of malignant cells get into the vascular bed:

- a smaller group (about 1%) – true malignant cells: cells with altered genotype and phenotype, which have autonomy, the capability for proliferation, prolonged circulation in the bloodstream and organisation of mature metastasis.
It is the true malignant cells, circulating in the blood stream, which examine the host-body seeking the most optimal tissues or target-organs, where those structural features remain that contribute to the formation of the "pre-metastasis" bed, and mark them: they release specific molecules (the spectrum of cytokines), which impact remotely on the fibroblast-like cells of

stroma of the tissue or of the target-organ and stimulate them to produce fibronectin;

- a larger group (99%) – conditionally malignant cells: the cells whose phenotype is altered under the influence of growth factors of the true malignant cells.
Conditionally malignant cells are more differentiated, less malignant, and not capable of proliferation and organisation of metastasis. Once got into the bloodstream, they die at various stages of circulation. Attempts to cultivate them in vitro have been unsuccessful. By their death, conditionally malignant cells can create in the bloodstream, and therefore in the host-body in general, a certain biochemical environment – the optimum for the development of the malignant process.

**Second stage** – exit of the haematopoietic stem cells out of the red bone marrow into the bloodstream and then into the tissues or target-organs:
- the primary malignant focus (malignant cells) releases into the bloodstream a special secret “Osteopontin”, which stimulates production of the haematopoietic stem cells in the red bone marrow and their release into the bloodstream;
- from the bloodstream the haematopoietic stem cells migrate to the already marked tissues or target-organs. Haematopoietic stem cells have integrins – surface receptors that have tropism to fibronectin, and thus they are looking for a place for future metastasis in the tissues or target-organs.

The first group of haematopoietic stem cells along the chemotaxis axes ends by the adhesion and formation of the “pre-metastasis” bed. Then a second group of haematopoietic stem cells comes, causing angioneogenesis and completing formation of the “pre-metastasis” bed. In this way, the haematopoietic stem cells can modify the normal cells of the microenvironment, with the purpose that they serve as structural components of the “pre-metastasis” bed.

**Third stage** - migration of a malignant stem cell and organisation of a mature metastasis: after the formation of the “pre-metastasis” bed, the migration of a malignant stem cell of the **second generation** goes from the vascular bed into the tissue or target-organ according to the following scheme: first, fixation of the cell to the vessel’s wall, then penetration beyond the vascular wall into the intercellular space and, finally,

penetration into the “pre-metastasis” bed. As a result of the proliferation of malignant cells and the influence of the microenvironment and the malignant progression, a mature metastasis is organised and the **third generation** of malignant stem cells emerges.

**Result**

***M.I. Davydov et al. (2002):*** *for most solid tumours the term of metastases’ emergence is 18-24 months from when the primary tumour has been “cured”.*

It is known that after radical treatment of the primary malignant focus, a “lucid interval” is observed. At this time there are no signs of metastases, but 1.5 – 2 years pass and metastases are detected. This shows that the mechanism of metastasis has already been “launched” by malignant cells of the primary focus, and, despite its radical surgical removal, the process has been continuing and ended with a mature metastasis.

A special feature of metastasis is the “favourite” tissues or target-organs, where the organisation of mature metastasis takes place. Metastatic affection of bones is one of the most frequent manifestations of malignant disease: breast, prostate, lung, kidney, thyroid, gastrointestinal tract, ovaries and other. Metastases in the liver frequently occur with cancer of the gastrointestinal tract.

**Thus**, organisation of the mature metastasis is a repetition of embryonic haematopoiesis and the “leading” role belongs to the primary malignant focus.

## 2.2 DEATH OF CELLS AND NON-CELLULAR STRUCTURES

The death of cells and non-cellular structures is a necessity conditioned by the law of evolution, it is an integral part of the progression of oncogenesis.

**Basis**

Cells and non-cellular structures in oncogenesis mostly die by necrosis. Necrosis is a pathological process that is characterised

by the impaired integrity of membranes, increase in the volume of the cell due to swelling of its cytoplasm and nucleus, DNA degradation, destruction of organelles, lysis of granules and the exit of cell content into the intercellular space with damage to adjacent cells (Fig. 22).

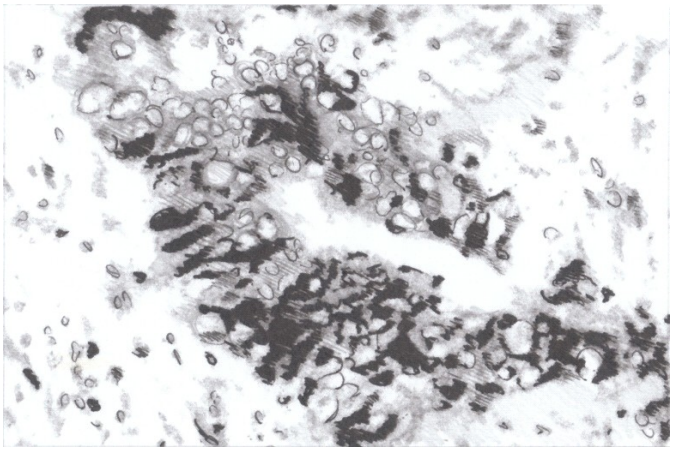


Fig. 22. Adenocarcinoma (I.A. Kraevskiy, A.V. Smolyaninova, D.S. Sarkisova, 1993, Vol. 1., p.137): necrosis of tumour cells.

Also, the phenomenon of apoptosis in cell structures is rarely observed. Apoptosis is the programmed death of a cell with the help of its internal mechanisms. It is initiated by extracellular or intracellular signals, under the influence of which the activation of proteases happens, which causes DNA fragmentation, condensation of the cell nucleus and the destruction of the cytoskeleton. This is followed by the fragmentation of the cytoplasm, with budding of vesicles surrounded by the membrane which are absorbed then by neighbouring cells and tissue macrophages (Fig. 23).

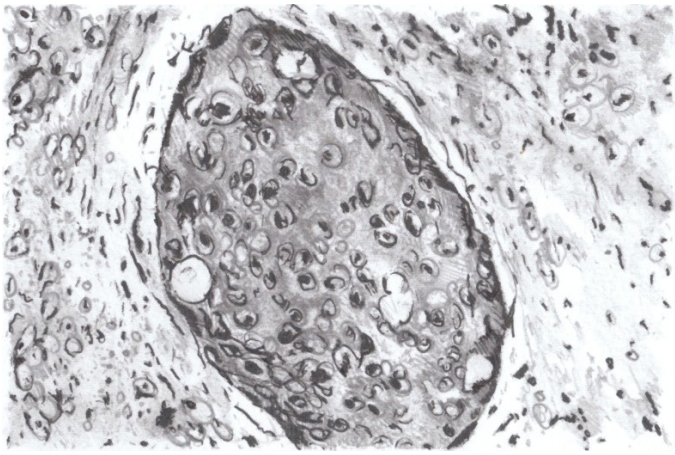


Fig. 23. Adenocarcinoma (I.A. Kraevskiy, A.V. Smolyaninova, D.S. Sarkisova, 1993, Vol. 1., p.141): apoptosis of tumour cells.

## Terms and localisation

The death of cells and non-cellular structures in oncogenesis is caused by abrupt disorders in blood circulation, by chemical and toxic agents etc.

1. FORMATION OF THE “PRE TUMOUR” BED

There are three groups of pathological changes in local tissues in the area of chronic inflammation, and the first of them is alteration.

Alteration means here a tissue injury: degenerative, destructive and necrobiotic phenomena. Primarily there is destruction of cells damaged during the inflammatory process: leukocytes, monocytes, macrophages and parenchymatous cells of local tissues. As a result of this the enzymes of lysosomes are released, which destroy tissues and make changes to paraplastic substances: separation of fibres, dissolution of collagen and elastic fibres, resorption of the basic substance of the bone and cartilage and the phenomenon of tissue amorphisation.

2. “GERMINATION” OF A MALIGNANT CELL

In red bone marrow under carcinogenic impact (ionising radiation, endo- and exo-carcinogens and viruses) genotypic changes happen in the nuclear DNA of a haematopoietic stem cell, and some of these cells die.

In the migration of a genotypic-changed tissue mononuclear cell from the red bone marrow to the isolated micro cavity of a chronic inflammation focus, a part of promonocytes and monocytes die. In this case, the mutant DNA molecules can be observed in the blood plasma.

When a genotypic-changed mononuclear cell gets into an isolated micro cavity containing an aggressive liquid in an

oxygen-free environment, the structural alterations of the cell membrane and “chemical evolution” in the cytoplasm occur, leading a part of these cells to their death.

A part of the genotypic-changed tissue mononuclear cells that already have structural alterations in the cell membrane and chemical changes in the cytoplasm, die during the actualisation of the mechanism of transformation into the primary malignant stem cell.

## 3. GROWTH AND DEVELOPMENT OF THE MALIGNANT PROCESS

Due to the proliferation of the “germinated” primary malignant stem cell within the limits of an isolated micro cavity, there develops a malignant “fetus” in which part of the present mononuclear cells, fibroblasts and part of the newly emerging malignant cells die.

With the exit of malignant stem cells outside the isolated micro cavity into the intercellular space and subsequent appositional and invasive growth, the volume of the primary malignant focus increases. Throughout this process, a mass death of normal and malignant cellular and non-cellular structures takes place. Dead normal cells remain unreplenished, while the death of malignant cells is continuously replenished by other malignant cells, which are newly formed by proliferation and appositional growth.

With the exit into the bloodstream, most malignant cells die immediately or during the circulation through the host-body. Mutant DNA molecules of tumour origin are identified in the blood plasma. Formation of the secondary (metastatic) focuses also does not happen without the death of cellular and non-cellular structures, which organises a mature metastasis.

### Mechanism

The death of normal cells and non-cellular structures in the area of chronic inflammation is due to significant physico-chemical changes of intercellular substance: acidosis and osmotic pressure increase, enzymatic-autocatalytic processes also increase, $CO_2$ grows as well as the quantity of K and Na ions in the tissues etc.

The death of malignant cells is mainly due to anaerobic glycolysis, which is used by them. This gives rise to the accumulation of a significant volume of lactic acid, which can reduce the pH level to a critical value, that in turn leads the cell to necrosis.

However, this mechanism also has positive aspects:
- an opportunity to block the mitochondria, and thus the production of cytokines leading the cell to the process of apoptosis;
- the death of a parts of the cell population results in stimulation and continuous replacement by other cells, which are newly formed by mitosis and/or amitosis;
- in the case of cells' apoptosis, the true malignant cell receives only very late the end-products of decomposition, while in the case of necrosis it gets much faster the large molecules, which can be used for energy production and as building materials for new protein compounds (catabolism);
- the death of conditionally malignant cells leads to the appearance of the optimal biochemical environment for the development of a malignant process in the blood, and thus throughout the host-body.

**Result**

1. The death of cellular and non-cellular structures in the organs and tissues due to necrosis, serves two important purposes:
- firstly, the supply of decay products as a source of energy and building material for malignant cells;
- secondly, the strengthening of local aseptic inflammation for the stimulation of its mediators and for organising a connective tissue stroma as the framework for a malignant neoplasm.

2. The death of malignant cells in the vascular bed due to necrosis, serves two important purposes:
- firstly, the supply of decay products as a source of energy and building material for malignant cells;
- secondly, creation in the blood, and thus throughout the whole host-body, of the optimal biochemical conditions for the development of the malignant process.

***3. Shabad L.M. et al. (1937):*** *the experiment proved the existence of blastomogenic substances in the body of patients who died of cancer – the theory of endogenous carcinogens*.

**Thus,** the death of cellular and non-cellular structures is conditioned by evolution, it is a necessary and integral part of the progression of oncogenesis. Parts of the decay products can be described as “seeds” of the future life of “germinated” and developing true malignant cells.

## 2.3 THE REDISTRIBUTION OF WATER

The redistribution of water in the body is a process conditioned by evolutionary development, and in various conditions it can manifest itself as a protective mechanism. However, this protective mechanism has not necessarily been designed to benefit the host-body. In each particular situation, compensatory or pathological processes occurring in the body can use the mechanism of water redistribution for their own purposes.

In the case where the pathological process manages to use the mechanism of water redistribution at the cellular and tissue level for its benefit, it will have an advantageous position, and its progression will lead to significant damage of vital organs and systems of the macro-organism.

### Characteristics of water

All biochemical processes in humans come down to biochemical reactions in aqueous solution – to metabolism. The cells of our body are floating in intercellular fluid. Movement in intercellular space does not stop for a second in normal tissues. Water largely determines the physical properties of the cell (volume, elasticity); it is involved in metabolism, transport of nutrients, oxygen, carbon dioxide and also in removing toxic substances from the body.

Water is:
- the electrolyte, which serves as the conduction system and is present inside the cell, in the extracellular space and in the blood;
- the universal solvent - all the biochemical reactions of the cell take place therein;
- an important participant in the thermoregulation of the organism.

## Water in the human body

In an adult's body, water accounts for 60-65% of the body weight and may be categorised into the intracellular and extracellular.

On average the volume of intracellular fluid is about 40% of the total body fluid weight. Most of the cells consist of water at 70-80% and it represents the sum of liquids in cells with different localisation, function and composition.

The volume of extracellular fluid is approximately 20-25% of the total body fluid weight and consists of the liquid part of the plasma (5% of the total body weight), interstitial fluid (15% of the total weight) and trans-cellular fluid (1-3% of the total weight): secretions of the gastro-intestinal tract, cerebrospinal, intraocular, pleural, peritoneal and synovial fluids.

Each liquid has its own, strictly set properties and characteristics. Extracellular and intracellular fluids are significantly different in composition and concentration of individual components, but the overall total concentration of osmotic active substances is about the same.

Table 2

The concentration of electrolytes and organic components in the body fluids in humans (averaged data from different sources)

| The components of body fluids | The concentration of substances in liquid sectors | | |
|---|---|---|---|
| | Blood plasma | Interstitial fluid | Intracellular fluid |
| Electrolytes, mmol/ l | | | |
| $Na^+$ | 135 - 142 | 144 | 10 |
| $K^+$ | 3.5-5.4 | 4.0 | 140 - 160 |
| $Ca^{2+}$ | 2.2-2.7 | 1.2 | 2 - 5 ($*10^{-4}$) |
| $Mg^{2+}$ | 0.8-1.6 | 0.7-1.0 | 13.5-58 |
| Cl- | 110 | 114 | 2-25 |
| $HCO_3$ | 27-29 | 30.5 | 8-10 |
| $HPO_4$ | 2.1-4.2 | 4.0-4.4 | 75-80 |
| $N_2PO_4$ | 2.1-4.2 | 4.0-4.4 | 75-80 |
| $SO_4$ | 1,1-2,2 | 2.0-2.4 | 4-40 |

| | | | |
|---|---|---|---|
| Protein, g / l | 20-50 | 1.0 | 160 - 550 |
| Glucose, g / l | 0.9 | - | 0-0.2 |
| Amino acids, g / l | 0.3 | - | 2.0 (?) |
| Cholesterol, g / l | 5.0 | - | 20 - 950 |
| Phospholipides, g / l | 5.0 | - | 20 - 950 |
| Neutral fats, g / l | 5.0 | - | 20 - 950 |
| pH | 7.36-7.4 | - | - |

**Water and the malignant process**

During all periods of emergence, growth and development of the malignant process at the cellular and tissue levels, the redistribution of water and alterations of its physical and chemical properties take place.

1. FORMATION OF THE "PRE TUMOUR" BED:

In the area of chronic inflammation the three groups of pathological changes of local tissues take place, and one of them is a consequently growing disorder of the microcirculation: a brief spasm of arterioles succeeded by dilated capillaries, arterioles and venules, stagnation and, finally, stasis of the blood and lymph circulation. As a result of difficulties of the blood outflow into the venous system, the liquid part and corpuscles of blood sequentially go out of the vessels into the inflamed tissue – exudation.

Exudation - a complex process, which is determined by an increase in blood pressure in the venous part of capillaries, increased permeability of capillary walls and increased osmotic and oncotic pressure in the focus of inflammation.

Exudates – form a pathological liquid of inflammatory origin, which has exited into the tissue. Serous inflammation is characterised by an accumulation of liquid exudates containing protein, various cell forms and products of their injuries.

Inflammatory oedema is formed due to the increase of adsorption processes, i.e. binding of water, proteins and salts by the tissues as a result of growth in them of the osmotic concentration,

change in the viscosity of released proteins and the phenomenon of coagulation when contacting denatured tissue surfaces. The basis of the binding of water is endosmotic and molecular imbibition of structural elements followed by their swelling and homogenisation (amorphisation), as well as cessation of the circulation in the tissue clefts.

## 2. “GERMINATION” OF A MALIGNANT CELL

In the area of the chronic inflammation a micro cavity isolated from the surrounding tissue forms, which is filled with a fluid of a specific chemical composition: an aggressive blend of enzymes, toxins, products of metabolism and cellular debris and inorganic ions; plus all of this is in the acidic oxygen-free environment. At the same time, the fluid inside the micro cavity contains water, electrolytes and proteins.

Under these conditions the “germination” of a primary malignant stem cell occurs, which contains 10% less water than normal somatic cells of the microenvironment. In addition, the water in the malignant cell is mainly in the nucleus. This feature gives some advantage to the malignant cell in the absorption of nutrients, and increases its ability to proliferate and resist hypoxia.

The main reason for the redistribution of water between the cell and the intercellular fluid is the increase in concentration of sodium in the extracellular space and consequently an increase of the osmotic mass concentration in the extracellular fluid. As the cell is separated from the extracellular fluid by the membrane, which is permeated by the protein structures – pores, easily penetrable by water - thereby in the presence of different concentrations of substances, water goes into the sector with the higher concentration of the solution.

## 3. GROWTH AND DEVELOPMENT OF THE MALIGNANT PROCESS

The growth of a malignant focus and the development of the malignant process are accompanied by changes in the functions of a range of organs and systems, with abnormalities of

microcirculation in the host-body and, as a consequence of this, accumulation of watery fluid – transudate.

Transudate or non-inflammatory effusion is an oedema fluid poorly filled with proteins, that is accumulated as a result of extravasation of the serum in the tissues and body cavities: in the pericardial cavity (hydropericardium), in the abdominal cavity (ascites), in the pleural cavity (pleurorrhea), in the cavity of the testicular membranes (hydrocele) and in the subcutaneous tissue (anasarca). Transudate is usually serous, rarely hemorrhagic or chylous. Sometimes in the peritoneal cavity there accumulates 8 to 30 litres of fluids or even more.

Optimisation of biochemical conditions for the development of the malignant process leads to disruption of blood and lymph circulation, water-salt metabolism and increased permeability of the vessel walls. As a result, a larger volume of fluid is maintained in the malignant focus compared with normal tissues of the environment. Moreover, the malignant process initiates a redistribution of water in the host-body – it creates artificial water pools or ponds in the natural cavities (pleural, abdominal etc.) and in the intercellular space of the individual parts of the body (oedema) or in the whole body (anasarca).

This gives rise to a paradoxical situation: in conditions of water retention in the organism, its dehydration is progressing. Hence there are various possible complications of the following nature: infectious, thrombotic, gastrointestinal (nausea and vomiting, hiccups, constipation, diarrhea, mucositis) and also intoxication and mental alterations (anxiety, depression, aggressive reactions, suicides).

Before the death of the host-body, the malignant process stimulates the rapid consumption of available reserve fluid, so sharp dehydration occurs, leading to catastrophic breaches in vital organs and systems.

**Thus**, the malignant cell and the malignant process stimulated by it, use the mechanism of water redistribution for their benefit, creating for themselves an advantageous position in relation to the host-organism.

# RÉSUMÉ

A clone of malignant cells is a “mafia clan”, which exploits the hormonal system and destroys the brilliant organisation of the organism from its inside.

# CONCLUSION

It seems possible to us to combine all the above in a simple, easy to understand universal theory, which is capable of reflecting the key provisions of a multistage mechanism for the emergence, growth and development of the malignant process, in which many of the accepted facts would find their place.

# MONONUCLEAR ONCOGENESIS

The path of mononuclear oncogenesis from the formation of a “pre-tumour” bed till the development of the malignant process as a self-contained system, is long and difficult. Definitely, the whole of this path develops according to a certain programme and has its own specific morphological, biochemical, immunological and other signs. It is not separated parts which have determined its emergence, but pre-tumour diseases of the organism in general and pathological alterations in local tissues in particular, which have created optimal conditions for its emergence, growth and development.

## 1. PROGRAMME

The programme of the emergence, growth and development of mononuclear oncogenesis in the host-body is composed of many determinate, evolutionarily conditioned and successive periods and stages, separated in time and space as well as of local alterations in the cell (nucleus, cell membrane and cytoplasm):

**Period I** (pre-cellular) – formation of the “pre tumour” bed.

**First stage** – emergence and development of **common diseases of the organism**, both basic and associated: non-specific alterations in tissues of the inflammatory, dystrophic and dishormonal nature; benign tumours; malformations; age-related changes etc.

**Second stage** – emergence and development of pre-tumour pathological **alterations of local tissues**: specific morphological, biochemical, immunological and other changes of local tissues in the area of chronic inflammation.

A “pre-tumour” bed is created in the form of an isolated micro cavity. Simultaneously, the chronic inflammation stimulates haematopoiesis – proliferation of the bone marrow mononuclear cells.

**Period II** – the “germination” of a malignant stem cell.

**First stage** (initiation) – in the red bone marrow, as a result of the stimulation of haematopoiesis and following the carcinogenic impact (ionising radiation, endo- and exo-carcinogens and viruses) a range of distinctive abnormalities of a pluripotent precursor of the progenitor of myelopoiesis occur, followed by the development into a monocyte shoot (II class) or a unipotent precursor of the progenitor of the monocytes (III class) according to the recessive sign. These abnormalities occur at the levels of gene, chromosome and genome: amplifications (increase of gene abundance), deletions, insertions, translocations, micro-mutations (point substitutions, micro-deletions and micro-insertions) etc.

An initiated cell emerges, which is a mononuclear cell having **genotypic alterations** in the nuclear DNA, while phenotypically it is a normal cell (promonocyte, monocyte).

**Second stage** (promotion) – in an isolated micro cavity of the focus of chronic inflammation, an aggressive fluid in an oxygen-free environment impacts the cell membrane and cytoplasm of a tissue genotypic-altered mononuclear cell (promonocyte, monocyte). Within the isolated micro cavity in conditions close to embryonic ones, there are proliferating cells which are fixed to the coat or are in a suspended state.

Structural alterations of the cell membrane occur, followed by breaching of the selective permeability for inorganic ions and also “chemical evolution” in the cytoplasm of the genotypic-altered mononuclear cell – **epigenetic changes**.

**Third stage** (the transformation mechanism itself) – in the isolated micro cavity **mitosis** of a tissue mononuclear cell (promonocyte or monocyte) takes place, which has genotypic and epigenetic alterations, transforming into the primary malignant stem cell. During mitosis implementation of the transformation mechanism occurs, as a continuous process consisting of two parts:

- **part one** – manifestation at the level of genotypic changes: return of the mononuclear cell during mitosis to the embryonic state and blocking the differentiation of daughter cells at the pluripotent or unipotent level, which is appropriate to the level at which the genotypic changes in the nuclear DNA of the bone marrow stem cell occur in haematopoiesis;

**- part two** – manifestation of the nature of genotypic alterations: a range of changes occurs in the nuclear DNA at the levels of gene, chromosome and genome: amplifications (increase of gene abundance), deletions, insertions, translocations, micro-mutations (point substitutions, micro-deletions and micro-insertions) etc.

An unstable active system “germinates” – a **primary malignant stem cell**, which retains many key abilities and capacities of the mother cell – the tissue mononuclear cell (promonocyte, monocyte), which has not fully left the embryonic state, but has acquired new abilities in its new life.

**Period III** - the growth and development of the malignant process.

**First stage** – **formation of the malignant “germ”**: due to the proliferation of the primary malignant stem cell, the accumulation of similar or homogeneous malignant cells, located within the coat of an isolated micro cavity, happens. Due to the expansive growth the size of the malignant “germ” may significantly increase.

**Second stage – organisation of the primary malignant focus**: when malignant cells exit beyond the isolated micro cavity into the intercellular space, followed by subsequent proliferation, appositional and invasive growth, organisation and increase of the primary malignant focus takes place.

**Third stage – organisation of the secondary malignant focus – metastasis**: invasive growth and angioneogenesis help the penetration of malignant cells into the bloodstream and the organisation of the secondary malignant focus – metastasis.

The malignant process, as a self-contained system, is capable of self-organisation and self-regulation. Throughout its growth and development it is accompanied by the deliberate death of cells and non-cellular structures, the redistribution of water, autonomous regulation, malignant progression, the growth of superiority, as well as the control and subjugation of the host-body.

## 2. PHASES

Phases of formation and the succession of generations of malignant stem cells in the case of mononuclear oncogenesis are appropriate to the phases of formation and the succession of generations of stem cells in the case of embryonic haematopoiesis.

The rendered comparative analysis of the stages of formation of stem cells' generations during embryonic haematopoiesis and mononuclear oncogenesis shows that the "germination" of a malignant stem cell, growth of the primary tumour and organisation of metastases are a perverse repetition of the basic stages of embryonic haematopoiesis during the postnatal development of the human body.

Table 3

Comparative analysis of the stages of formation and succession of the stem cells' generations in the cases of embryonic haematopoiesis and mononuclear oncogenesis.

| Embryonic haematopoiesis | Mononuclear oncogenesis |
|---|---|
| The first phase – the first generation of stem cells | |
| **Mesoblastic** – in the wall of an embryo's yolk sac a "birth" of blood stem cells occurs – the **first generation**. | **Cytoblastic** – in the isolated micro cavity formed like a kind of embryo's yolk sac, the "germination" of a malignant stem cell occurs. Due to proliferation, the mass accumulates and formation of the **first generation** of the malignant stem cells takes place. |
| The second phase – the second generation of stem cells | |
| **Hepatolienal** – the blood stem cells exit from the yolk sac and colonize the liver, which becomes the main body of embryonic haematopoiesis. The **second generation** of the blood stem cells is formed therein. Then, the stem cells of the liver blood colonize thymus, spleen and lymph nodes. | **Primary-focal** – the malignant stem cells exit beyond the isolated micro cavity into the intercellular space and colonize it. Due to proliferation, appositional and invasive growth they organise a primary focus, where the **second generation** of the malignant stem cells is formed. |
| The third phase - the third and subsequent generations of stem cells | |
| **Medullar (marrow)** – the blood stem cells colonize the red bone marrow, where their **third generation** is formed – this is the final phase of the embryonic haematopoiesis. | **Secondary-focal or metastatic** – due to the invasive growth and angioneogenesis the malignant stem cells penetrate into the bloodstream and colonize the lymph nodes, the bone marrow, liver, lungs etc., followed by organisation of the secondary malignant focus – mature metastasis, where the **third generation** of the malignant stem cells is formed. Later, subsequent |

| | |
|---|---|
| | generations of the malignant stem cells may form. |

## 3. PROTOTYPES

Mononuclear oncogenesis uses known processes and structural organisations as prototypes, according to the principle “all this has already been in the body, but in another time, in another place and with other cellular and non-cellular elements”.

The prototype of a “pre-tumour” bed, presented as an isolated micro cavity, is the structural organisation and functioning of an embryo’s yolk sac. In this case the conditions close to embryonic ones occur inside the micro cavity and the “germinated” malignant stem cell conditionally repeats the beginning of embryonic haematopoiesis.

The prototype of the structure and functioning of the primary malignant focus is the structural organisation and functioning of the red bone marrow. In this case the primary malignant focus is presented as an independent structural unit, which contains all the classic signs of a tissue, has an autonomous reproduction and the ability to spread in the host-body.

The prototype of the relationship between the structural elements that constitute the malignant process, as well as their relationship with the host-body, is the hormonal system. In this case the malignant process is presented as a self-contained system, which is characterised by its own control of proliferation, differentiation and maturation of malignant cells, their spread and metastasis, as well as the subjugation of vital organs and systems of the host.

The prototype of the emergence, growth and development of the malignant process is embryonic haematopoiesis. But the malignant process in a perverted form conditionally repeats all phases of formation and succession of the stem cells’ generations of embryonic haematopoiesis.

## 4. BASIS

The basis for the growth and development of mononuclear oncogenesis is a malignant stem cell, and the basis for the

“germination” of a malignant stem cell is the return of a tissue mononuclear cell, which has genotypic and epigenetic alterations, during mitosis to its embryonic state, blocking differentiation at the pluripotent or unipotent level, and transformation.

In the red bone marrow, as a result of the carcinogenic impact (ionising radiation, endo- and exo-carcinogens and viruses) a range of distinctive abnormalities of a pluripotent precursor of the progenitor of myelopoiesis occur, followed by the development into a monocyte shoot (II class) or a unipotent precursor of the progenitor of monocytes (III class) according to the recessive sign. These abnormalities occur at the levels of gene, chromosome and genome: amplifications (increase of gene abundance), deletions, insertions, translocations, micro-mutations (point substitutions, micro-deletions and micro-insertions) etc. As a result, in the host-body an initiated cell appears – the mononuclear cell, which has genotypic alterations in the nuclear DNA, while phenotypically it is presented as a normal cell (promonocyte, monocyte).

The initiated cell, while maintaining the stages of its development in the red bone marrow and the vascular bed, exits into the focus of chronic inflammation and finally gets into the isolated micro cavity. Here, surrounded by an aggressive oxygen-free environment, its structural changes are evolved in the cell membrane and the chemical changes in its cytoplasm – epigenetic changes.

A tissue mononuclear cell emerges, which has genotypic and epigenetic alterations – this is a potential precursor of the primary malignant stem cell of solid tumours. On the face of it such a mononuclear cell is a normal cell while it is in the inter-phase, but as soon as it proceeds to mitosis, all its alterations will become obvious and manifest themselves.

It is known that in tissues every promonocyte and monocyte transforms into an organ-specific and tissue-specific macrophage. The transformation is a series of cell divisions, when its phenotypic alterations occur successively under the influence of the microenvironment.

A promonocyte or monocyte having genotypic and epigenetic alterations, attempts to transform itself into a macrophage and

begins the process of mitosis, during which it returns to the embryonic state. However, after mitosis the level of genotypic alterations shows itself; thereby occurs a block of differentiation of the daughter cells and the transformation, during which the nature of genotypic alterations of the nuclear DNA shows itself.

As the result of this an unstable active system is “germinated”: the malignant stem cell, which retains many basic abilities and capacities of the mother cell – the tissue mononuclear cell (promonocyte, monocytes), which has not fully left the embryonic state and which acquires new capabilities of its new life: the possibility of uncontrolled division, autonomous regulation, immortality of the population etc.

The malignant stem cell is a proliferating somatic cell having a certain level of potency, which corresponds to the level at which the genotypic alterations in the bone marrow cells have occurred during haematopoiesis:

1. If the genotypic alterations in the bone marrow cell have occurred at the level of a pluripotent precursor of the progenitor of myelopoiesis, followed by the development into a monocyte shoot (II class), then the tissue mononuclear cell transforms into a pluripotent malignant stem cell, which has clear phenotypic heterogeneity and the possibility of the emergence of a “cell-chimera” with multiple differentiation.

2. If the genotypic alterations in the bone marrow cell have occurred at the level of a unipotent precursor of the progenitor of monocytes (III class), then the tissue mononuclear cell transforms into a unipotent malignant stem cell, which has minimal phenotypic heterogeneity.

The “germinated” malignant stem cell creates by proliferation a clone of the true malignant cells. Under the influence of growth factors secreted by the true malignant cells into the intercellular space, “conditionally malignant cells” emerge – phenotype-altered tectorial or glandular epithelium. The true malignant cells are the trigger, the regulator and the activator of the growth and development of the malignant process.

## 5. MECHANISM

The mechanism of the emergence, growth and development of mononuclear oncogenesis is a complex process, where each subsequent action is the result of the previous one, each action has its own peculiarities, and oncogenesis may be terminated at any of them due to various causes.

Pre-tumour diseases of the organism and pathological alterations of the local tissues in the area of chronic inflammation contribute to formation of an isolated micro cavity as the future “pre-tumour” bed. Isolated from the microenvironment, the micro cavity contains aggressive specific fluid in an oxygen-free environment.

Mediators of inflammation initiate an additional requirement for specific tissue immunocompetent cells, whereby haematopoiesis is stimulated and the production of mononuclear cells in the red bone marrow intensifies, because they do not form a bone marrow reserve.

During the accelerated production of the bone marrow mononuclear cells and under the carcinogenic impact, genotypic alterations in the nuclear DNA of haematopoietic stem cells occur; they are of various levels of potency according to the recessive sign. After the exit into the tissues, the mononuclear cells having genotypic alterations of nuclear DNA penetrate into an isolated micro cavity, where they undergo the impact of aggressive fluid in an oxygen-free environment – the epigenetic alterations appear.

In the process of mitosis of the mononuclear cell which has genotypic and epigenetic alterations, a primary malignant stem cell “germinates”, which divides then forms similar or homogeneous malignant cells and a monoclonal malignant “germ” – a clone of malignant cells within the coat of the isolated micro cavity.

The subsequent division of malignant cells leads to an increase in their critical mass (quantity) accompanied by their increased malignancy (malignant progression), that contributes to the destruction of the coat of the isolated micro cavity. The exit of malignant cells into intercellular space and involvement of the stroma of an organ or a tissue in the malignant process, means the beginning of a primary malignant focus.

The malignant focus increases in its size due to active proliferation, appositional and invasive growth. Active penetration of the true malignant cells through the tissue barriers (invasive growth), as well as the stimulation of growth of the blood vessels (angioneogenesis) facilitate the penetration of malignant cells into the bloodstream, and organisation of the secondary malignant focus (metastasis).

## 6. FEATURES

The peculiarity of the formation of the “pre tumour” bed is the obligatory presence of pre-tumour diseases of the organism in general and pre-tumour pathological alterations of the local tissues in particular. Emergence of the malignant process in normal healthy tissues is impossible.

The peculiarity of the preparation of a cell for transformation into the malignant cell is that this process is multistage. So each stage has its own features determining the possibility of continuing the process, from which the decisive genotypic and epigenetic alterations can emerge.

The peculiarity of the “germination” of the primary malignant stem cell is the obligatory entry of the cell into mitosis, which has genotypic and epigenetic alterations. Only during **mitosis** does the implementation of the transformation mechanism occur and the level and the nature of genotypic alterations manifest themselves. In this case the mechanism of transformation takes place in the isolated micro cavity and, thus disengages from the influence and control of the local tissues.

The peculiarity of the growth of the tumour focus is its autonomy and its ability for self-organisation and self-regulation. Using the host-body as the basis for its own development, the malignant process subjugates normal cellular and non-cellular structures of the vital organs and systems.

The peculiarity of the mechanism of penetration of a mononuclear cell from the vascular bed into the tissue, and of the malignant cells from the primary focus into the vascular bed, is the use of the same area of microcirculation – postcapillary and venule. Speaking in images, “through whichever ‘door’ the cells go out, through the same they return”.

The peculiarity of the development of the malignant process is a perverse repetition of embryonic haematopoiesis. The host-body unconsciously “switches on” a false version of strengthening its ability to survive at the expense of maintaining some aging organs. In reality, this way is dead-locked and the mechanism fatal.

## 7. RESULT

As a result of the pre-tumour diseases of the organism in general and the pathological alterations in local tissues in particular, the formation of an isolated micro cavity as a “pre-tumour” bed takes place. However, the pre-tumour alterations of local tissues are only necessary preparations to create conditions in which the genotypic- and epigenetic-altered cell can be transformed into a malignant stem cell.

In the red bone marrow, as a result of carcinogenic impacts, there occur various genetic alterations of the nuclear DNA of a pluripotent precursor-cell of the progenitor of myelopoiesis, followed by its development into a monocyte shoot, or a unipotent precursor-cell of the progenitor of monocytes, according to the recessive sign. These alterations do not lead to a breach of the cell’s differentiation, but they are inherited by the more mature cells – promonocyte and monocyte. The result is an initiated cell – the genotypic-altered mononuclear cell.

In an isolated micro cavity of a chronic inflammation focus, the initiated cells are exposed to aggressive fluid in an oxygen-free environment. This leads to structural alterations of the cell membrane with breach of its selective permeability and to “chemical evolution” in the cellular cytoplasm. As a result, the genotypic-altered mononuclear cell gets epigenetic alterations.

As the result of mitosis of the genotypic- and epigenetic-altered mononuclear cell, the mechanism of transformation into a malignant stem cell is launched and a primary malignant stem cell is “germinated”, which then divides, forming a clone of the same type of homogeneous malignant cells. The result of this is a malignant “germ” – a clone of malignant cells within the coat of the isolated micro cavity.

The consequence of the exit of malignant cells beyond the coat of the isolated micro cavity and involvement of the stroma of the microenvironment in the malignant process, is the organisation of the primary malignant focus. The increase in it is the effect of the active proliferation, appositional and invasive growth of malignant cells, but the penetration into surrounding tissues is the result of the invasive growth of the true malignant cells.

As the result of active penetration of the true malignant cells through the tissue barriers (invasive growth), as well as stimulation of growth of the blood vessels (angioneogenesis), the malignant cells penetrate into the bloodstream and participate in organisation of the secondary malignant focus (metastasis).

The effect of the ability to self-control the proliferation, differentiation and maturation of the malignant cells, their spread throughout the host-body and organisation of metastasis, as well as the consequence of the influence on vital organs and systems of the host-body and their subsequent “subjugation” is that the malignant process develops as a self-contained system.

## 8. KEY PROVISIONS

**Provision 1:** the precursor-cell of the primary malignant stem cell of solid tumours is a tissue mononuclear cell (promonocyte, monocyte) having genotypic and epigenetic alterations.

**Provision 2:** the basis for the “germination” of a malignant stem cell is the return of the tissue mononuclear cell having genotypic and epigenetic alterations, to the embryonic state during mitosis, blocking the differentiation at the pluripotent or unipotent level, and transformation.

**Provision 3:** the mechanism of “germination” of the malignant stem cell is a complex multistage process, when under a carcinogenic impact the genotypic alterations of a bone marrow mononuclear cell subsequently occur; then the epigenetic alterations of the same cell, already a tissue mononuclear cell, under the influence of the “super circumstances” of the isolated micro cavity; and mitosis is the starting point for the realization of these alterations.

**Provision 4:** polymorphism of the malignant cells is due to a variety of options for their “germination”, maturation and differentiation, as well as their own evolution and the impact of the microenvironment.

**Provision 5:** the malignant focus is an independent structural and functional formation having its peculiarities, and conditionally repeating the structural organisation and functioning of the red bone marrow.

**Provision 6:** the phases of formation and the succession of generations of malignant stem cells conditionally repeat the phases of formation and the succession of generations of the embryonic haematopoiesis stem cells.

**Provision 7:** the malignant process, as a self-contained system, is capable of self-organisation and self-regulation, and many evolutionary-conditioned mechanisms are in the basis of its origin and development.

## 9. PROSPECTS FOR DIAGNOSIS AND TREATMENT

Contemporary diagnostic methods are based on crusted ideas about the aetiology and pathogenesis of malignant diseases. Mononuclear oncogenesis, as a theory, gives a completely new understanding of the earliest alterations in certain precursor-cells of malignant stem cells as well as of the morphological changes in local tissues. Based on these ideas new diagnostic techniques and methods can be suggested.

With regard to treatment of malignant diseases, it is certain that it is completely impossible to transform an existing malignant cell into a normal cell. However, it can be asserted that all of the advantages of the emergence, growth and development of the malignant process are at the same time its shortcomings. So it is possible to succeed in this way.

# RÉSUMÉ

The above concept has not yet been formulated in its final form by anyone, so in the available literature we did not find contenders for its joint authorship.

# BIBLIOGRAPHY


- Abe R., Donnelly S.C., Peng T. et al. Peripheral Blood Fibrocytes: Differentiation Pathway and Migration to Wound Sites. The Journal of Immunology. 2001; 166: 7556-62.
- Bjerkvig R., Tysnes B.B., Aboody K.S., Najbauer J., Terzis A.J. et al. Opinion: the origin of the cancer stem cell: current controversies and new insights. Nat. Rev. Cancer 2005; 5(11): 899-904.
- Carcinogenesis. These 532 abstracts provide an excellent introduction to current research. Proc. Am. Assoc. Cancer Res. 34: 99-187, 1993.
- Cheatle G.L., Cutler M. Tumors of the breast. – Philadelphia: J.B. Lippincott, 1931.
- Conheim I. Vorlesungen uber eligemine Pathologie. Berlin Hirchwald. 1877, 2. p.601.
- Cornil A.V. Les Tumeurs du Sein. Paris: Libraire Germer Bailliere and Company, 1908.
- De Palma M., Vinneri M.A., Roca C & Naldini L. Targeting exogenous genes to tumour angiogenesis by transplantation of genetically modified hematopoietic cells. Nature Med. 2003; 9: 789-795.
- Druker B.J., Mamon H.J., Roberts T.M.: Oncogenes, growth factors, and signal transduction. N. Engl. J. Med. 321: 1383-1391, 1989.
- Finean J.B., The molecular organization of cell membranes, «Progress in Biophysics and Molecular Biology», 1966, v. 16, p. 143—70.
- Hay A.: Testing times for the tests. Nature 350: 555-556, 1991.
- Houghton J., Stoicov C., Nomura S., et al. Gastric cancer originating from bone marrow-derived cells. Science 2004; 306: 1568-15710.
- Koerner J., Nesic D., Romero J.D. et al. Equine Peripheral Blood-Derived Progenitors in Comparison to Bone Marrow-Derived Mesenchymal Stem Cells. Stem Cells. 2006; 24(6): 1613-9.

- Kucia M., Reca R., Miekus K., et al. Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. Stem Cells 2005; 23(7): 879-94.
- Kuznetsov S.A., Mankani M.H., Leet A.I. et al. Circulating Connective Tissue Precursors: Extreme Rarity in Humans and Chondrogenic Potential in Guinea Pigs. Stem Cells. 2007; 25(7): 1830-9.
- Lyden D. et al. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumour angiogenesis and growth. Nature Med. 2001; 7: 1194–1201.
- Maximow A. Über die Entwickelung der Blut- und Bindegewebszellen beim Säugetierembryo. Fol. haem., 1907, 4.– P. 611.
- Moore G.E., Palmer Q.N.: Money causes cancer, ban it. J.A.M.A. 238: 397, 1977.
- Neeson P., Thurlow P., Jamieson, G. & Bradley C. Lymphocyte-facilitated tumour cell adhesion to endothelial cells: the role of high affinity leukocyte integrins. Pathology 2003; 35: 50–55.
- Neve E.F., Neve A.: Kangri-burn cancer. Br. Med. J. 2: 1255-1256, 1923.
- Passeque E., Jamleson C. H. M., Ailles L. E., Weissman I. L. Normal and leukemic hematopoiesis: Are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? PNAS, 2003, 100 (Suppl. 1).– P. 11842–11849.
- Potter V. Recent trends in cancer biochemistry - Canad. Cancer Conf. 1968. No.8 p. 9-30.
- Potter V.R. 1978, Br. J. Cancer, 38, 1-23.
- Quan T.E., Cowper S.E., Bucala R. The role of circulating fibrocytes in fibrosis. Curr. Rheumatol. Rep. 2006; 8(2): 145-50.
- Schmidt M., Sun G., Stacey M.A. et al. Identification of Circulating Fibrocytes as Precursors of Bronchial Myofibroblasts in Asthma. The Journal of Immunology. 2003; 171: 380-9.
- Singh S.K., Hawkins C., Clarke I.D., et al. Identification of human brain tumour initiating cells. Nature 2004; 432: 396-401
- Wynn R.F., Hart C.A. Corradi-Perini C., et al. A small proportion of mesenchymal stem cells strongly express functionally active CXCR4 receptor capable of promoting migration to bone marrow. Blood, 2004. 104(9): 2643-5.

PUBLISHED IN RUSSIAN


- Абелев Г.И., Альтштейн А.Д., Дейгман Г.И., Дыбан П.А. и др. Опухолевый рост как проблема биологии развития. М., «Наука», 1979, 244 стр.
- Абрамов М.Г. Гематологический атлас. М., Медицина, 1985, 344 стр.
- Алмазов И.В., Сутулов Л.С. Атлас по гистологии и эмбриологии. М., Медицина, 1978, 544 стр.
- Атлас клеток крови и костного мозга. /Под редакцией Г.И.Козинца/. «Триада-Х», М., 1998, 160 стр.
- Афанасьев Б.В., Алмазов В.А. Родоначальные кроветворные клетки человека. Л., «Наука», 1985, 204 стр.
- Белохвостов А.С., Новик А.А. Роль молекулярно-генетических исследований в диагностике солидных опухолей. «Вопросы онкологии». Т.45, №6, 1999, стр. 599-606.
- Бжадуг О.Б., Гривцова Л.Ю., Тупицин Н.Н., Тюляндин С.А. Циркулирующие опухолевые клетки в крови больных местнораспространенным и диссеминированным раком молочной железы. «Вестник российского онкологического научного центра им.Н.Н.Блохина», Т. 18, № 3, 2007, стр.19-21.
- Билибин Д.П., Бабиченко И.И., Ходорович Н.А. Патофизиологические и патоморфологические аспекты острого и хронического воспаления. М., Изд. Российского университета дружбы народов. 2003, 35 стр.
- Бочков Н.П. Клиническая генетика. Учебник. М., ГЭОТАР-мед, 2002.
- Быкорез А.И., Рубенчик Б.М. Причины рака: факты и гипотезы. Киев, Наукова Думка, 1987, 118 стр.
- Вахтин Ю.Б., Пинчук В.К., Швембергер И.Н., Бутенко З.А. Клонально-селекционная концепция опухолевого роста. Киев. Наукова Думка, 1987, 215 стр.
- Владимирская Е.Б. Биологические основы противоопухолевой терапии. М., Агат-Мед, 2001, 110 стр.
- Воробьев А.И. Руководство по гистологии. М., Медицина, Т.1, 1985, 447 стр.
- Галанкин В.Н., Токмаков А.М. Проблемы воспаления с позиций теории и клиники. М., Изд. Университета дружбы народов, 1991, 120 стр.

- Гарин А.М., Базин И.С. Десять наиболее распространенных злокачественных опухолей. М., Агенство «КМН», 2006, 266 стр.
- Герасимов И.Г., Попандопуло А.Г. Оценка жизнеспособности клеток по их морфометрическим параметрам на примере культивируемых фибробластов. Цитология. 2007, Т.49, № 3, стр. 204-209.
- Гистология, цитология и эмбриология. /Под редакцией Ю.И.Афанасьева, Н.А.Юриной/. М., Медицина, 2002, 737 стр.
- Гистология. /Под редакцией Э.Г.Улумбекова, проф. Ю.А.Челышева/. М., ГЭОТАР-МЕД, 2001, 672 стр.
- Горбунова В.Н., Имянитов Е.Н. Генетика и канцерогенез. Методическое пособие. С-Пб, СПбГПМА, 2007, 24 стр.
- Гранов А.М., Шутко А.Н. Парадоксы злокачественного роста и тканевой несовместимости. С-Пб, Изд. «Гиппократ», 2002, 223 с.
- Григорян А.С. Роль миграционной оси SDF-1-CXCR4 в хоуминге клеток-предшественников злокачественных опухолей. Клеточная трансплантология и тканевая инженерия, 2006. 4 (6): 32-7.
- Грин Н., Стаут У., Тейлор Д. Биология. М., «Мир», Т.1, 1990, 367 стр.
- Давыдов М.И., Демидов Л.В., Поляков Б.И. Основы современной онкологии. М., 2002, 237 стр.
- Данилов Р.К., Климов А.А., Боровая Т.Г. Гистология. ЭЛБИ-СПБ, С-Пб, 2003, 360 стр.
- Жолондз М.Я. Рак. Мифы, теория, профилактика. Комплект, С.-П., 1998, 335 стр.
- Зайчик А.Ш., Чурилов Л.П. Механизмы развития болезней и синдромов. С-Пб, «ЭЛБИ-СПб», 2005, 507 стр.
- Зенгбуш П. Молекулярная и клеточная биология. Т.3, М., «Мир», 1982, 343 стр.
- Злокачественные опухоли. Клиническое руководство. /Под редакцией Н.Н.Петрова, С.А.Холдина/. Л., МЕДГИЗ, 1952, Т.2. 944 стр.
- Иванов К.П. Основы энергетики организма. Т.1, Л., «Наука», 1990, 307 стр.
- Ивашкин В.Т., Васильев В.Ю., Северин Е.С. Уровни регуляции функциональной активности органов и тканей. Л., «Наука», 1987, 272 стр.
- Иващенко Ю.Д., Быкорез А.И. Полипептидные факторы и канцерогенез. Киев, Наукова думка, 1990, 192 стр.

- Имянитов Е.Н., Хансон К.П. Молекулярная онкология: клинические аспекты. С-Пб, СПбМАПО, 2007, 213 стр.
- Калишевская Т.М., Коломина С.М., Кудряшов Б.А. Свертывающая и противосвертывающая система крови и их значение при развитии злокачественных новообразований. М., Издательство Московского Университета, 1992, 167 стр.
- Канцерогенез. /Под ред. Д.Г.Заридзе/. М., «Научный мир». 2000, 420 стр.
- Киселев Ф.Л., Павлиш О.А., Татосян А.Г. Молекулярные основы канцерогенеза у человека. М., Медицина, 1990, 316 стр.
- Кнорре А.Г. Эмбриональный гистогенез. 1971.
- Краевский А.Н., Смольянников А.В., Саркисрв Д.С. Патологоанатомическая диагностика опухолей человека. Т.1, М., Медицина, 1993, 559 стр.
- Кривчик А.А. Патофизиологические аспекты опухолевого роста. Минск, «Вышэйшая школа», 1987, 143 стр.
- Луговская С.А., Почтарь М.Е. Гематологический атлас. М., «Триада», 2004, 227 стр.
- Макаренко Н.П. Фиброзно-кистозная болезнь. «Онкология», Т. 13, № 13, 2005, стр. 875-877.
- Мари Э.Вуд, Пол.А.Банн. Секреты гематологии и онкологии. Перевод с английского. М., Из-во «БИНОМ», 2001, 559 стр.
- Маянский Д.Н. Хроническое воспаление. М., Медицина, 1991, 271 стр.
- Меклер Л. Б. Механизмы индукции опухолей в свете общей теории онкогенеза. – Успехи современной биологии, 1978, 85, вып.1.
- Морозкина Т.С. Энергетический обмен и питание при злокачественных новообразованиях. Минск, «Беларусь», 1989, 190 стр.
- Патологическая физиология. /Под редакцией А. Д. Адо, Л. М. Ишимовой/. М.: Медицина, 1980.
- Патологоанатомическая диагностика опухолей человека./Под редакцией И.А.Краевского, А.В.Смольянинова, Д.С.Саркисова/. Т. 1-2, М., Медицина, 1993.
- Петерсон Б.Е., Чиссова В.И. Ранняя онкологическая патология. М., Медицина, 1985, 316 стр.
- Петрова А.С. Цитологическая диагностика опухолей и предопухолевых процессов. М., Медицина, 1985, 302 стр.

- Практическая онкология: избранные лекции. /Под редакцией С.А.Тюляндина, В.М.Моисеенко/. С-Пб., «Центр ТОММ», 2004, 784 стр.
- Райхлин Н.Т., Давид Р., Лапиш К. Ультраструктура опухолей человека. Руководство для диагностики. М., Медицина, 1981, 550 стр.
- Раны и раневая инфекция. /Под редакцией М.И.Кузин, Б.М.Костюченок/. М., медицина, 1990, 591 стр.
- Репин В.С., Ржанинова А.А., Шаменков Д.А. Эмбриональные стволовые клетки: фундаментальная биология и медицина. М., «Реметэкс», 2002, 176 стр.
- Терентьева Н.А., Алясова А.В., Шахов Б.Е. Лимфома Ходжкина. Нижний Новгород. Издательство «НижГМА», 2008, 431 стр.
- Терещенко И.П., Кашулина А.П. Патофизиологические аспекты злокачественного роста. М., Медицина, 1983, 255 стр.
- Токин Б.П. Общая эмбриология. М., Медицина, 1987, 480 стр.
- Трапезников Н.Н., Соловьев Ю.Н., Шингаров Г.Х. Методологические вопросы изучения онкогенеза. М., Медицина, 1988, 206 стр.
- Трумэн Д. Биохимия клеточной дифференцировки. Перевод с английского. М., Мир, 1976, 188 стр.
- Франкфурт О.С. Клеточный цикл в опухолях. М., Медицина, 1975, 170 стр.
- Ходосова И.А. Биохимические аспекты канцерогенеза. М., Наука, 1976, 205 стр.
- Хэм А., Кормак Д. Гистология. Т. 1-5. Перевод с английского. М., «Мир», 1982.
- Шапот В.С. Биохимические аспекты опухолевого роста. М., Медицина, 1975, 304 стр.
- Шимке Р.Н. Генетика и рак у человека. Перевод с английского. М., Медицина, 1981, 183 стр.
- Щелкунов С.И. Цитологический и гистологический анализ развития нормальных и малигнизированных структур. Л., Медицина, 1971, 399 стр.
- Эйген М. Самоорганизация материи и эволюция биологических макромолекул. Перевод с английского. М., Мир, 1973, 216 стр.